Resolution of spatial ambiguity by the hippocampal place system

Dorothy W. Overington

Thesis submitted to University College London in consideration

for the Degree of Doctor of Philosophy in Neuroscience

Declaration

I, Dorothy Overington, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Dorothy Overington

Acknowledgements

This thesis would never have been possible without the love, friendship, support, and patience of so many people who deserve my thanks.

I would firstly like to thank my supervisors Kate Jeffery and Robin Ali for their guidance and support throughout my PhD. Kate, in particular, has been an excellent source of experimental ideas and advice as well as offering unwavering support and patience in some difficult times.

For funding my PhD, I would like to thank the BBSRC Doctoral Training Programme, and particular thanks go to Nadine Mogford for her sound advice and for bossing the bureaucratic ups and downs of my studentship.

I am also indebted to the Bogue Fellowship scheme at UCL, for funding 5 incredible months of science and life in San Francisco. I came back from California renewed and refreshed in my love of brains, ready to tackle my final experiments and thesis.

For my time in San Francisco, I must principally thank Loren Frank and Jai Yu. Working with you both enriched me as a scientist, and I learnt more than I ever thought possible in such a short time. Thanks also to the Frank Lab (especially Irene Grossrubatcher) for making my time in San Francisco exciting and far from lonely.

Thanks, of course, to all my colleagues at the IBN, whose friendship have made these 4 years fly by even in those inevitable slumps (James S and Roddy, thanks so much for your help with getting the computer to understand my thoughts!). Special thanks also to Fabio, you are one of my dearest friends and I can safely say this thesis would not be what it is today without your constant encouragement and pick-me-ups.

Thanks too, to the Institute of Ophthalmology gang as a whole, and in particular to Matteo for always having faith in me, and Kate & Nikki for great discussions and the most fun of play times.

To my friends in London (especially Cat, Min, Matt, Shumin, Cliff, Rach, Ray, Han), thanks for putting up with my terrible schedule and keeping me happy and somewhat sane. Keep being yourselves and you'll be great.

Finally, my famery (Mum, Emily, Adam). Words cannot describe my gratitude for you all in my life. Please never stop pushing me to achieve my best, supporting me when I'm at rock bottom, and keep believing in me. I know I'm constantly preoccupied and not always the best communicator, but it's done now and I love you guys.

Abstract

External space is coded in the brain by a network of spatially modulated neurons (including place, grid, border and head direction cells), known as the ‘cognitive map’. This internal map allows flexible and efficient navigation through the external world. These neurons use both self-motion and visual landmark information to update their spatial activity and form an accurate representation of space. Sometimes, the spatial meaning of a landmark can be ambiguous, e.g. when it can be approached from different directions (for example, a tree on the border between two fields). In such cases context information, such as odour, colour or texture, can provide clues to separate one environment from another.

Recent work has shown that head direction (HD) cells in the retrosplenial cortex can use these non-metric cues to resolve visually symmetrical spaces with directional landmark ambiguity. In this study, we asked whether animals can also use these non-metric cues to guide their behaviour, in this case in order to solve spatial tasks across multi-compartment space.

Here we show that, behaviourally, rodents can correctly encode relative object positions in visually ambiguous space, and can resolve the directional ambiguity of two visually symmetrical spaces based only on odour information. Electrophysiological recordings of hippocampal place and anterior thalamus HD cells confirmed that both cell types can use odour-context information to discriminate these spaces; therefore, we tested potential involvement of the HD system by temporarily inactivating the anterior thalamus with an awake muscimol infusion. In the behavioural task, HD-disrupted animals show impairment in task performance compared to sham but retain response to novelty. Overall, these results indicate that rodents can use odour-context information to resolve directional ambiguity in otherwise identical multi-compartment environments, and suggest an involvement of the HD system in this process.

Table of Contents

Table of Figures

Table of Tables

Abbreviations

ADN	anterodorsal thalamic nucleus
ATN	anterior thalamic nuclei
AVN	anteroventral thalamic nucleus
CA	cornu ammonis region (e.g. CA1 / CA2)
DG	dentate gyrus
DTN	dorsal tegmental nucleus of Gudden
EC	entorhinal cortex
HD	head direction
HF	hippocampal formation
HPC	hippocampus
LDN	lateral dorsal nucleus
lEC	lateral entorhinal cortex
LFP	local field potential
LMN	lateral mammillary nucleus
mEC	medial entorhinal cortex
PER	perirhinal cortex
PHR	parahippocampal region
POR	postrhinal cortex
PoS	postsubiculum
RSC	retrosplenial cortex
SUB	subiculum

Chapter 1 - General Introduction

The ability to navigate the world is a vital part of survival for all mobile animals; it is necessary for finding food and mates, avoiding predators and finding the way home. The processes underlying accurate navigation and spatial awareness were once thought to be the result of learnt stimulus-response associations, known as Thorndike's Law of Effect (Thorndike, 1911). As these associations were thought to be inflexible, this view led to the notion that navigation was also rigid and that animals could only use learnt associations to guide themselves to goals. This, however, rules out any possibility of animals being able to adapt to unexpected change in their environments such as obstacles in their planned routes or the appearance of shortcuts.

Edward Tolman began to challenge the stimulus-response association theory in the mid twentieth century and suggested that rats possess an internal representation of external space (Tolman, 1948). This idea that animals possess a so-called 'cognitive map', allowing flexible and adaptive navigation, has since become the focus of understanding the processes behind how animals find their way in the world (Tolman, 1948).

When navigating an environment, landmarks can be unreliable if there is ambiguity in the direction from which they are encountered. For example, a direction stating "the church is to the left of the Empire State Building" is devoid of context for where left actually is for the person interpreting the direction. Direction without context brings ambiguity. With this in mind, the overarching question of this thesis relates to how the brain, and this cognitive map, makes use of contextual information in the environment to resolve spatial (in particular for this work, directional) ambiguity.

1.1 Tolman's Cognitive Map

The cognitive map is a representation of the external environment inside the brain, integrating information about locations and salient environmental features which can then be used in a flexible manner to inform animal navigation. The classic behavioural experiment that first cemented the possibility of an internal spatial representation was conducted on an elevated maze named the 'sunburst maze' (Tolman et al., 1946). In this task (Figure 1.1A), rats were trained to run from a start platform along a single path toward a goal location. In the test phase, rats were reintroduced to a modified version of the previous maze that, instead of having a single path to the goal, had a number of different paths radiating out from the platform in all directions (Figure 1.1B). When exposed to this new platform, the rats would preferentially choose the shortest direct path to the goal despite having never explored that path before. Tolman deduced from

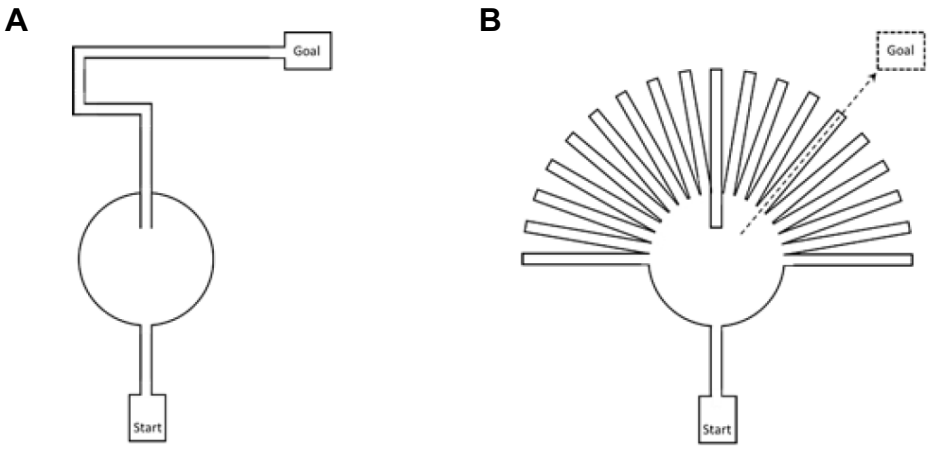


Figure 1.1: The 'sunburst' maze used by Tolman *et al.* (1946). (A) Rats are initially trained to navigate from a start box to a rewarded goal via a single path. (B) At test, rats are given a choice of 18 different alleys. Only one alley is correct and rewarded, and corresponds to the straight line trajectory to the previous location of the reward chamber (labelled with an arrow).

this behaviour that navigation could not be inflexible, as Thorndike had previously posited (Thorndike, 1911), as the rats could correctly choose a shortcut to the goal location and use a location-route relationship that they had never experienced; this indicated the presence of an internally stored representation of space that was "allocentric" or world-centred. However it has since been argued that Tolman's use of a light over the goal location may have provided an external cue that could guide the animal's behaviour through a learned stimulus-response association (between the light and reward; O'Keefe and Nadel, 1978).

Much of Tolman's further research provided additional support for the presence of a cognitive map. One of his seminal experiments showed that rats can learn without any motivation or clear stimulus-response associations, and thus revealed that performance is not necessarily a direct indicator of learning (Tolman and Honzik, 1930). This was termed 'latent learning'; a process by which an animal learns without any motivation, reward, or even any obvious improvement in task performance (a task-related improvement would occur if the animal were to become rewarded for a goal at a later time). The experiment used a complicated maze consisting of 14 interconnected T mazes (Tolman and Honzik, 1930) (Figure 1.2A): rats were required to reach a goal location in the maze, and could do this only after following a series of correct left or right turns. Three groups of rats were tested on the maze: one group was trained to navigate the maze and find food reward at the goal location on each trial; a second group navigated the maze but no food reward was ever present; and a third group of rats navigated the maze with no food reward for 10 days, after which food reward at the goal became available. Unsurprisingly the task time taken (task latency) for the constantly rewarded group decreased steadily from the first day (shown here through

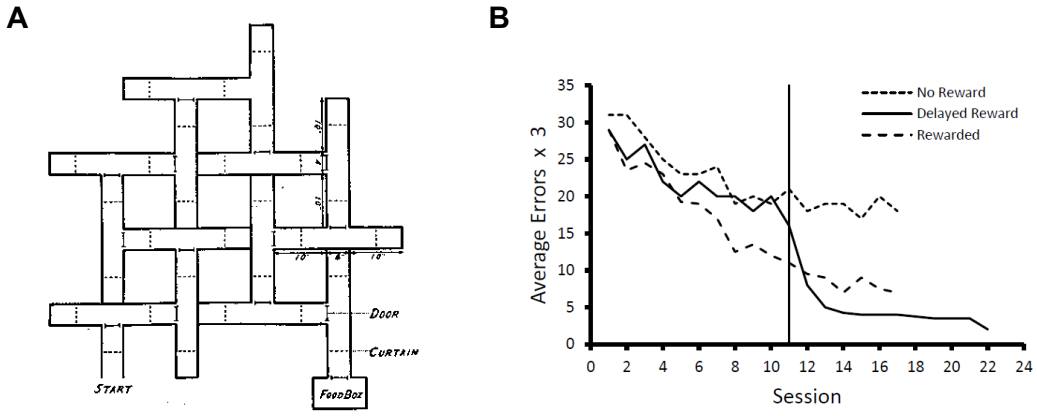


Figure 1.2: Latent learning in rats. (A) The complicated 14 interconnected T maze used by Tolman & Honzik, 1930: rats were required to reach a goal location in the environment by remembering a correct series of left or right turns. (B) Graph showing the average number of errors for the three different experimental groups; the vertical line marks the point when the delayed reward group began to receive food reward.

decline in average number of errors made, Figure 1.2B), where in the no-reward condition there was no decrease in task latency at all. The third group, however, gave the unexpected result: these animals performed similarly to the unrewarded group for the first 10 days, then immediately following the introduction of the reward on the 11th day there was a sharp drop in task latency to an even higher degree than the constantly rewarded animals. This result indicated that even when there was no reward to motivate the animals and they appeared to be 'bad' at solving the task, they were in fact latently developing an internal representation of the maze (or cognitive map) that could be rapidly utilised once the goal location was reinforced.

In addition to the aforementioned 'sunburst maze' task (Figure 1.1), Tolman, Ritchie and Kalish (1946) also compared place and response learning in a simple plus maze. Here, there were two starting locations and two possible goal locations. Rats were either trained in a 'response' group where they were required to always make a particular turn (right or left) to the goal location from the start, or trained in a 'place' group where they were required to always go to a 'goal location' in relation to external cues such that the path to the goal differed depending on the start location. The authors found that animals in the 'place' group learnt the task more readily than their 'response' group counterparts; this supported the idea that animals preferentially use place learning strategies and integrate external information, consistent with the proposal of a 'cognitive map'.

1.1.1 Looking forward from Tolman

Tolman's cognitive map theory forms the basis of many of the experiments in this thesis, and has been the foundation for behavioural paradigms to the current day. All of the results from the series of experiments conducted by Tolman point towards a flexible and adaptive system inside the rodent brain that can use all navigational cues in the

environment with varying reliance depending on the demands of the task and perceived reliability. As a behaviourist, Tolman's cognitive map was based on observation of the animal's actions and there was no mention of how and where such a complex system could exist in the brain. In the following years the search for such a structure began, and many studies started to point strongly towards a candidate structure: the hippocampus.

1.2 The Hippocampus as a Cognitive Map

One of the first big steps towards understanding where the foundations of the cognitive map could be found in the brain came from neurological studies in humans, and then rodents, where links to memory and spatial cognition were identified.

Research by Scoville & Milner (1957) on a group of patients with severe neurological and psychiatric impairments led to the proposal that the hippocampus is vital in the formation of new memories (and also generally important to episodic memory of 'what', 'where' and 'when' aspects of events, later described by Tulving, 1983). One particular patient, known as patient H.M. (Henry Molaison), had been diagnosed with severe epilepsy that could not be controlled by drugs, so underwent a bilateral temporal medial lobe resection to alleviate his symptoms. This surgery involved removal of a portion of the temporal lobe, including parts of the hippocampus, entorhinal cortices and amygdala, from both sides of the brain (Corkin, 2002). Post-surgery, H.M no longer had any symptoms of epilepsy but presented instead with a complete loss of the ability to form new memories (anterograde amnesia) as well as a temporally graded loss in memories prior to surgery (retrograde amnesia; memories more distant from the time of surgery were recalled better) (Scoville and Milner, 1957). Although at the time scientists could not identify which structure within the lobe was the cause of H.M.'s dramatic memory loss, there was now ample foundation for further study and, for the first time, there was direct evidence for a relationship between the hippocampus and memory.

Much work developed upon these foundations from the 1970s onwards, with the introduction of electrophysiological recordings in rodents. In seminal work by O'Keefe & Dostrovsky (1971), *in vivo* electrophysiology was used to record the activity of single neurons in the hippocampus of rats while the animals were awake and freely exploring an environment. These recordings led to the discovery of neurons in the hippocampus that would fire when the animal was in particular places in the environment. These neurons, now known as 'place cells', suggested that the hippocampus was the neural

basis of Tolman's cognitive map (O'Keefe and Nadel, 1978) as well as being critical to memory processes.

Following the discovery of place cells, the technique of single neuron electrophysiology has become one of the leading and most commonly used techniques for understanding how neural activity relates to behaviour, especially in the field of how the brain represents space. The technique has also provided discoveries of additional neurons that support the positional coding of place cells in the neural cognitive map: a subset of neurons that are directionally modulated, known as head direction cells (Ranck, 1984); neurons which fire with multiple, evenly-spaced firing locations over an environment, known as grid cells (Hafting et al., 2005); and neurons which respond maximally to borders or boundaries in an environment, known as border or boundary vector cells (Lever et al., 2009; Solstad et al., 2008). Taken together, these sets of cells are thought to provide positional, directional, boundary and odometry information (Marozzi and Jeffery, 2012) required to inform behaviour as part of the cognitive map proposed by Tolman.

However, recognising physical spaces as separate can be difficult if their salient visual features are largely similar. For example: a wooded area has stretches of land where the only features present are many of the same type of trees or plants, and hospitals or office buildings often have many similar looking rooms and corridors. It is therefore useful to represent the geometry and spatial aspects of a space in relation to the context in which the space is experienced; here, the 'spatial context' refers to non-metric sensory features of the animal's environment like smell, colour, contrast (light/dark), and texture. Processing the 'spatial context' of an environment is advantageous for survival because it can aid navigation in times of spatial uncertainty, and allow the same environment to be represented differently under different conditions.

It has previously been shown on both a behavioural and neuronal level that 'spatial context' can be used to inform spatial representation: behaving rats are able to remember the particular context in which they encountered an object (Eacott and Norman, 2004), while spatially modulated neurons have been shown to respond to changes in non-metric features (Anderson and Jeffery, 2003; Anderson et al., 2006; Jacob et al., 2016; Marozzi et al., 2015). These points will be expanded in Chapters 3 and 5.

Building on these ideas, the experiments in this thesis examine whether animals can use context to aid interpretation of directionally ambiguous information. Specifically, if

animals can solve a task in a directionally ambiguous environment using odour context cues or directional cues (or a combination of both) (Chapter 5), how neurons of the cognitive map respond to this directionally ambiguous environment (Chapter 6), and whether the head direction system is necessary for the behaviour of animals in the aforementioned task (Chapter 7).

Before this set of experiments are reported, a selected literature review of the neural mechanisms that underlie navigation will be presented, followed by a detailed account of neural representation in both clearly different spaces and in spaces with ambiguity.

Chapter 2 - Neural Basis of the Cognitive Map

Research into the cause of H.M.'s amnesia led to the suggestion that the hippocampus was vital to producing complete memories of events (Scoville and Milner, 1957) and, with the discovery of place cells, on to the view that the structure was also important to spatial navigation (O'Keefe and Nadel, 1978). O'Keefe & Nadel's book, 'The Hippocampus as a Cognitive Map', delved into this importance based on observations of the effects of lesions and damages to the structure in animals as well as their electrophysiological recordings of spatially tuned neurons. However it was emphasised that while the hippocampus may be the core of the cognitive map, it is clear that it many of the surrounding structures in the hippocampal formation are necessary for accurate spatial representation (O'Keefe and Nadel, 1978).

To explore how the neurons of the cognitive map respond to non-metric stimuli, the experiments in this thesis examine and manipulate the circuits and information flow between the hippocampus and its connected areas. It is thus pertinent to first present an overview of these brain areas contributing to the cognitive map, and their form and function. Unless otherwise stated, experimental findings here refer to the rodent brain (specifically the rat) and anatomical data is summarised from the following book chapters and reviews: Amaral & Lavenex, 2007; Burwell & Agster, 2008; van Strien et al., 2009.

2.1 The Hippocampus

The term hippocampus was coined from the Greek, meaning 'seahorse', by the anatomist Arantius in 1587. This name reflects the C-shape of the structure in each hemisphere; two hippocampi are joined at the anterior end by the hippocampal commissure, separating towards the posterior of the brain (Figure 2.1A).

Three distinct subregions of the hippocampal formation (HF) can be distinguished: the dentate gyrus (DG); the hippocampus proper (consisting of the three 'cornu ammonis' regions, CA3, CA2 and CA1); and the subiculum (SUB). All of these regions have a trilaminar organisation, of which the nomenclature differs by region but the roles are fairly consistent: a deep layer, comprising a mixture of afferent and efferent fibres and interneurons; a more superficial cell layer, where densely-packed principal neurons are found; and the most superficial molecular layer which is predominantly cell-free (Figure 2.1B). In the DG, the deep layer (named the hilus) is enclosed in a U shape by the principal granule cell layer and stratum moleculare. In the CA and SUB regions, the

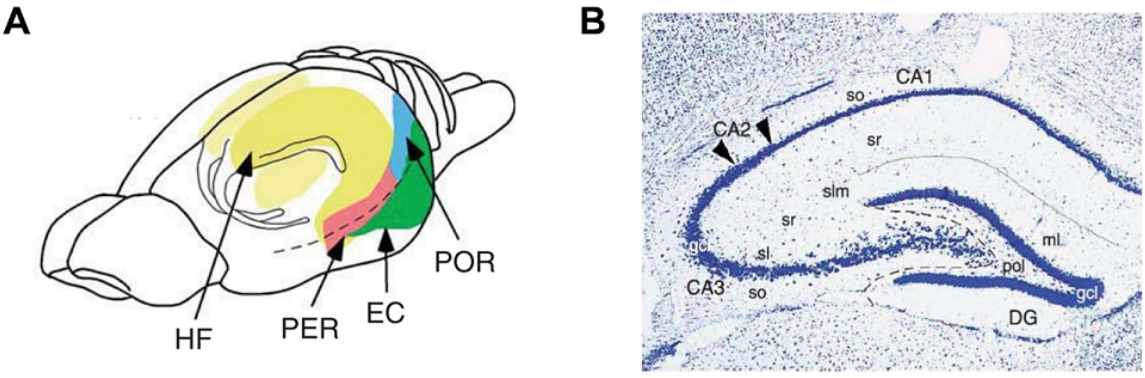


Figure 2.1: Anatomy of the hippocampal formation within the rat brain. (A) Line drawing of the HF and parahippocampal regions. The C-shaped HF is highlighted in yellow, while the perirhinal cortex (PER), postrhinal cortex (POR) and entorhinal cortex (EC) of the parahippocampal area are also shown. Dotted line marks the rhinal sulcus that forms a prominent anatomical landmark for histological slicing, dorsally separating the EC from POR/PER. (B) Laminar organisation of the HF from a photomicrograph of a nissl-stained coronal section. The trilaminar structures of the DG and CA fields are shown. Layers of the DG are (from deep to superficial): the polymorphous layer / hilus (pol), granule cell layer (gcl) and molecular layer (ml). CA fields contain (from deep to superficial): the stratum oriens (so), pyramidal cell layer (gcl), stratum radiatum (sr) and stratum lacunosum moleculare (slm). CA3 also contains the stratum lucidum (sl). The dashed line marks the border between CA3 and DG, the solid line the border between DG and CA1, and the black arrows represent the boundaries of CA2. [Images adapted from Burwell & Agster, 2008].

deep layer is referred to as the stratum oriens, next to which sits the pyramidal cell layer and then the molecular layer(s). Uniquely, the molecular layer of the CA regions is subdivided into a number of additional layers. In CA3, there are three sublayers: the stratum lucidum, which receives mossy fibre axon inputs from the DG; the stratum radiatum, containing the apical dendrites of neurons located in the CA3 pyramidal layer; and the most superficial stratum lacunosum moleculare, containing the apical tufts of the apical dendrites and perforant path fibres. The lamination in CA2 and CA1 is similar to this, except the stratum lucidum is not present.

The parahippocampal region (‘support to the core’, as noted by O’Keefe & Nadel, 1978) lies adjacent to the HF (Figure 2.1A), bordering the subiculum, and comprises five subregions: the presubiculum, parasubiculum, entorhinal cortex (EC), perirhinal cortex (PER), and postrhinal cortex (POR). The simplified view of connectivity between the HF and the parahippocampal region holds that there are three main intrinsic connections forming a ‘tri-synaptic loop’: the peforant path, the mossy fibres, and the Schaffer collaterals (Amaral and Witter, 1989; Witter and Amaral, 1995). These connections are excitatory via glutamate signalling and largely unidirectional (or non-reciprocal); unidirectional connectivity is unusual compared to the surrounding cortex, suggesting that the tri-synaptic loop has a specialised function.

The main input to the HF originates in the superficial layers of the EC (layers II and III), with the majority unidirectionally projecting onto DG granule cells via the perforant path and a small input directly to CA1. These DG granule cells give rise to distinctive axons,

known as mossy fibres, which provide a major input to pyramidal cells of CA3; this projection, again, is unidirectional. The pyramidal cells of CA3 then project unidirectionally on to pyramidal cells of CA1 via the Schaffer collateral system. Unlike the DG input to CA3, the Schaffer collateral axons projecting from CA3 to CA1 are topographically organised along the septotemporal axis; CA3 cells closer to the DG project to CA1 cells septal to their location and CA3 cells closer to CA1 project to cells more temporally located. Finally, to close the loop, information from CA1 is projected back to the deep layers of the EC (especially layer V) both directly and indirectly through the subiculum. Though the subiculum also projects onto the pre- and parasubicum, the more notable onward projections from the trisynaptic loop are from EC, where information is relayed out and back to the cortex. The trisynaptic loop as explained above is detailed in Figure 2.2, but it is important to note that this is an oversimplification: several back projections have in fact been reported, for example from CA3 to temporal DG (Li et al., 1994) and from CA1 back to CA3; direct inputs from superficial EC to the understudied CA2 region also exist (Chevaleyre and Siegelbaum, 2010), from which CA2 axons then project to the superficial layers of CA1 and proximal CA3 (Mercer et al., 2007).

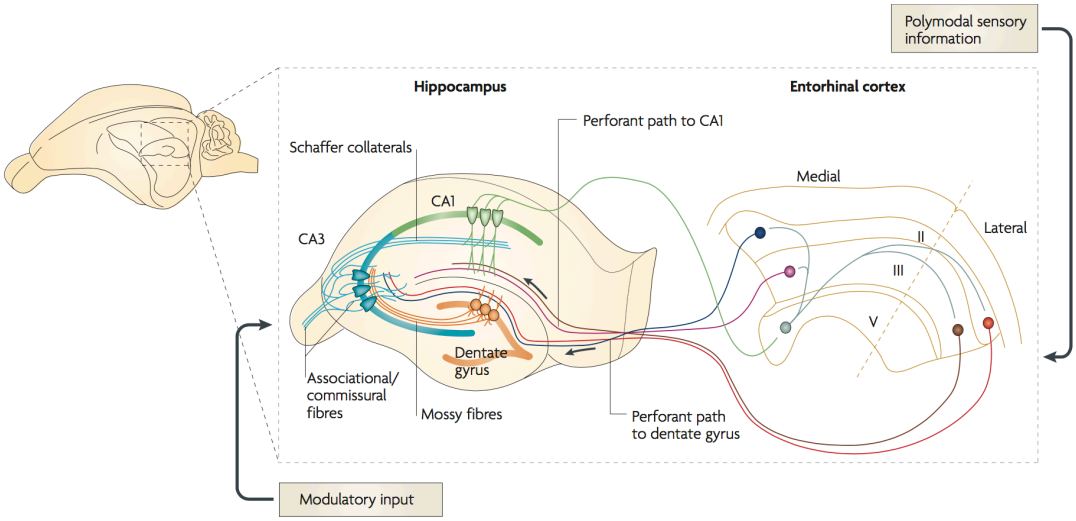


Figure 2.2: Simplified circuitry of the hippocampal formation. Diagram detailing the 'tri-synaptic loop' from EC to the HF. Neurons in EC layer II/III project to DG via the perforant path. Neurons in layer II/III of the lateral EC also project to CA1 and subiculum via the perforant path. From the DG, granule cells project to CA3 through mossy fibre axons. Pyramidal neurons in CA3 then project to CA1 (and SUB) via Schaffer collaterals. Information is then relayed back to the deep layers of the EC directly from CA1 and indirectly from CA1 via SUB. [Reproduced from Neves et al., 2008]

There are fairly few inputs to the HF from other brain areas, as the majority of synaptic input is generated within the HF itself. Of the few, inputs from the parahippocampal region are of particular importance, partially due to the role of the EC as a 'gateway' to the HF. This EC input is important to this study as it allows information from areas carrying directional and olfactory information to enter the HF. There are also notable inputs from subcortical areas such as the thalamus via the fornix, amygdaloid complex and brainstem (mainly locus coeruleus), as well as others from the medial septum, diagonal band of Broca, and the prefrontal cortex via nucleus reuniens. The amygdaloid complex input derives mainly from the basal nucleus, projecting to CA1, CA3 and SUB, and is hypothesised to provide information about the animal's emotional state to the HF. Reciprocal projections from medial septum and diagonal band of Broca carry strong cholinergic and GABAergic input to the DG and CA3, and weaker input to CA1 and SUB, and are critical to the generation of theta rhythm in the HF; theta rhythm is a prominent pattern shown in the hippocampal local field potential (LFP) that is associated with information processing and awake behaviours.

2.2 The Hippocampus and Place Cells

As briefly explored in Chapter 1, hippocampal 'place cells' were first described in the early 1970's (O'Keefe and Dostrovsky, 1971) and were the first evidence that there may be a neural basis for an allocentric 'cognitive map' allowing for flexible navigation as posited by Tolman et al. (1946). The name 'place cell' was coined due to the fact that these neurons maximally fire action potentials in a specific location in an environment (known as a 'place field') and are virtually silent everywhere else (Figure 2.3). These hippocampal place cells are pyramidal cells (Henze et al., 2000), found in the DG, CA1, CA2 and CA3 regions of the hippocampus (Lu et al., 2015; Mankin et al., 2015; Muller and Kubie, 1987; O'Keefe and Dostrovsky, 1971). In addition to the rat, place cells have been confirmed in monkeys (Rolls et al., 1989), mice (Tonegawa et al., 1996), pigeons (Bingman et al., 2006), bats (Ulanovsky and Moss, 2007) and humans (Ekstrom et al., 2003).

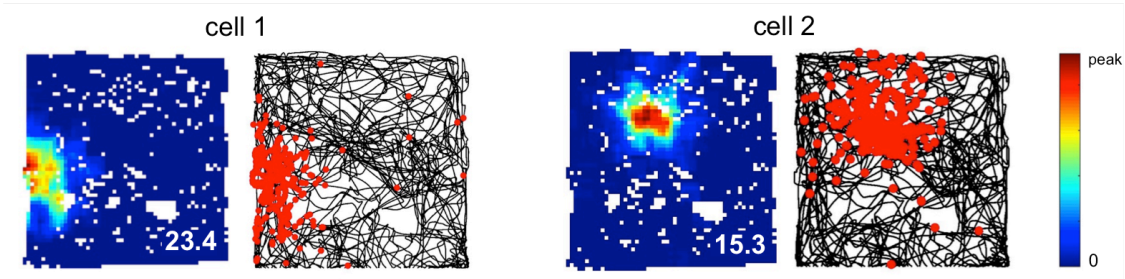


Figure 2.3: Example place cells recorded from rat hippocampus. Both cells were simultaneously recorded by the author (D. Overington) from the hippocampus of a freely moving rat in a 1mx1m square environment. Spike plot (right image for each cell) - black line represents the path of the animal through the environment, while superimposed red dots represent threshold-passing spiking events. This information can be alternatively represented as a rate map (left image for each cell), where 'hot' colours are maximal firing in Hz and 'cold' colours are minimal firing in Hz (shown by the scale bar). Peak firing rates (Hz) of each cell are written in white in the bottom left of the rate maps.

2.2.1 General properties

While the background-firing rate of place cells is extremely low (effectively zero), they rapidly begin to fire complex spikes as soon as the animal enters a place field. Complex spikes are bursts of 2-6 action potentials, separated by up to 6ms, which successively decrease in amplitude within the burst (Ranck, 1973). Peak firing rates (calculated as averaging the spikes fired in a spatial bin and dividing by the time spent in that bin, see section 4.4.3.4) of place cells range from ~40 Hz for a strongly firing cell to 1 Hz for extremely weak place cells (Muller, 1996). Note that for experimental datasets in this thesis, a threshold of 1 Hz was used to select place cells, and cells firing below this, no matter their spatial activity, were excluded. Different place cells recorded simultaneously are likely to have place fields that are equally distributed around the whole environment (Muller and Kubie, 1987), but possibly occur more often near walls or boundaries (Hetherington and Shapiro, 1997). Place field locations have been shown to be stable when the animal is returned to the same environment, persisting for days and even weeks (Lever et al., 2002; Thompson and Best, 1990). However, recent Ca^{2+} imaging studies looking simultaneously at thousands of CA1 pyramidal cells have shown dynamic place coding such that the ensemble of place cells active each day included a unique subset of cells (Ziv et al., 2013). This study also found that there was a 15-25% overlap between any two of these subsets that retained the same place fields, sufficing to preserve the accurate and stable spatial representation over weeks previously described. This stability is such that, after an initial exploration period, firing properties of a place cell population can be used to accurately predict the location of an animal (Wilson and McNaughton, 1993).

In addition to variation in peak firing rate between cells, there is also variation of firing rates within a given place field. Place fields can be described as a two-dimensional Gaussian tuning curve (O'Keefe and Burgess, 1996) such that the peak rate is likely to

occur at the centre of the field and decrease closer to the edges (Figure 2.3). In an open field, place cells are also omnidirectional; this means that the cell will fire independent of the direction the animal enters the field from, and during any behaviour of the animal. However, geometric properties of an environment (*e.g.* on a linear track) can influence place cells to develop strong directional tuning (McNaughton et al., 1983; O'Keefe and Recce, 1993), such that a cell may only fire when the rat enters the field from a particular direction. This type of trajectory-based directionality is not 100% consistent, so some similar but unique trajectories will not elicit firing in the same way (Fenton et al., 2010). Interestingly, there is more variability in firing rates than would be expected (from Poisson noise) on a single pass through a place field, for example cases when particular passes through a place field may elicit 10 action potentials, while a highly similar trajectory at another time may not prompt place cell firing at all. This phenomemon, known as 'over-dispersion', possibly reflects incorporation of unrelated information about the environment and was found to decrease with increased attention state of the animal (Fenton et al., 2010).

Alongside rate coding, place cell firing also has a relationship with the phase of theta oscillations in the local field potential. When the animal runs through a place field, the action potentials fired by the place cell tend to occur at progressively earlier phases of the ongoing local theta cycle (O'Keefe and Recce, 1993); this is called 'phase precession'. As phase precession correlates mainly with a rat's position in a given place field and has no bearing on firing rate, it suggests that this phenomenon can also add additional information about the rat's location within a field (Huxter et al., 2003; Jensen and Lisman, 2000).

2.2.2 Sensory influences on place cell firing

Experiments have shown that animals can use external cues (*e.g.* visual landmark information) as well as internal information generated from self-motion (*e.g.* proprioceptive, vestibular or motor efference copy information; Etienne and Jeffery, 2004) to refine their representations of a given environment. In turn, place cells can use and encode these inputs to update their firing and create an accurate representation of current space.

Early studies showed that place fields in an environment are anchored to distal landmark cues, to the extent that if the landmarks are rotated then the firing fields rotate accordingly (Muller and Kubie, 1987; O'Keefe and Conway, 1978; Yoganarasimha and Knierim, 2005). When proximal cues are rotated while distal cues remain stable, place fields also remain stable (Cressant et al., 1997), suggesting that

distal cues are more reliable landmarks for the animal. However, if a distal landmark becomes unreliable (Knierim et al., 1995; Jeffery and O'Keefe, 1999) or there are several proximal and distal cues available (Shapiro et al., 1997), then place cells have been found to respond to proximal cues just as much as distal ones. These findings suggest flexibility in place cell response to sensory input depending on the situation.

Despite these data above, place cells continue to fire after all salient cues have been removed from an environment (Muller and Kubie, 1987), are stable in darkness (Quirk et al., 1990; O'Keefe and Conway, 1978), and are present in rats that were blinded shortly after birth (Save et al., 1998). This suggests that while visual landmark information may provide salient input for place cells, they must also be influenced by other sensory modalities (*e.g.* tactile, olfactory and auditory cues) as well as internally generated information. These other sensory inputs, as well as any influence altering them may have on activity of place cells, will be discussed further in Chapter 3.

Animals can use information generated from their own movement to keep track of their position in an environment using a process termed 'path integration' (Mittelstaedt and Mittelstaedt, 1980; Etienne and Jeffery, 2004). In the complete absence of salient visual and olfactory information, self-generated information can be used to update place cell activity, but the process of path integration is prone to error accumulation and place fields are unstable (Save et al., 2000). However, blind rats have similar place fields to sighted rats if proximal cues (*e.g.* objects in an environment) are present (Cressant et al., 1997; Hill and Best, 1981; Save et al., 1998). These results suggest that place cells are not reliably able to keep track of animal's position in an environment without some external input; if reliant on path integration alone, place cells are still active but just prone to error. The process of path integration is dependent on a functional hippocampus in rats (Maaswinkel et al., 1999; Whishaw et al., 2001; Kim et al., 2013), lending more strength to the hypothesis that the hippocampus is indeed the core of the neural cognitive map.

2.3 The Entorhinal Cortex

The main input to the hippocampus, the EC can be divided in two along the mediolateral axis based on morphology and connectivity of its neurons: the medial entorhinal cortex (mEC) and lateral entorhinal cortex (lEC). These areas communicate differently with the hippocampus and surrounding cortical areas, making the sub-area distinction functionally relevant (Burwell and Agster, 2008). Both mEC and lEC are six-layered structures comprising two largely acellular layers (layer I and IV) and four cellular layers (layers II, III, V, and VI). These can be grouped into 'superficial' (layers I-

III) and ‘deep’ (IV-VI), based on the presence of particular cell types, cell layers or white matter (Canto et al., 2008). Layer II mainly contains stellate cells, thought to be putative grid cells in the mEC (Burgalossi et al., 2011), and pyramidal cells; it is the principal origin of the perforant path to the DG and CA3 regions of the HPC. Layer II also produces many collaterals that project to the superficial EC layers only (Köhler, 1986), providing known excitatory connections between the stellate cells of the mEC (Kumar et al., 2007). Layer III principally contains pyramidal cells that project to CA1 and SUB of the HPC, produce collaterals that innervate superficial EC layers, and send dendrites to layer I to receive inputs from sparse stellate cells, horizontal cells and GABAergic neurons there (Witter et al., 1989). Layer IV, though mainly acellular, does contain some fusiform and pyramidal cell bodies whose dendrites project to layer I and axons reach deep into the white matter. The superficial part of layer V contains large pyramidal neurons, whose apical dendrites project to layer I and II, with the axons of these cells projecting to the deep white matter and additional collaterals going on to innervate all layers of the EC. The deeper portion of layer V contains pyramidal cells, horizontal cells and atypical multipolar neurons whose dendrites extend from the EC to the subiculum; this suggests that layer V contains projection neurons to the DG and the HPC via SUB. Layer VI also contains a variety of cell types that influence other cells in both the deep and superficial layers, while also projecting to the deep white matter. Interneurons (mostly GABAergic) are present in all layers of the EC, but are most prominent in the superficial layers.

The EC provides the HPC with sensory information from the cortex both directly (Witter, 1993; Dickson et al., 2000) and indirectly via the perirhinal and postrhinal cortices (Burwell, 2000). Visuospatial information arising from cortical regions such as retrosplenial cortex projects to the postrhinal cortex before entering the DG via the mEC and the medial perforant path (Canto et al., 2008). In parallel, other sensory information (e.g. olfactory) from regions such as piriform cortex projects to the perirhinal cortex before entering the DG via the lEC and the lateral perforant path. These findings, in addition to the fact that spatially firing grid cells (described in the following section) are found in the mEC, while lEC does not appear to show strong correlates for any spatial property (Hargreaves et al., 2005; Yoganarasimha et al., 2011), have led to the suggestion that the mEC is more involved in processing spatial information, while the lEC processes non-spatial ‘contextual’ information alongside it (Burwell, 2000; Knierim, 2006; Manns and Eichenbaum, 2006; Deshmukh and Knierim, 2011; Van Cauter et al., 2013).

2.4 The Medial Entorhinal Cortex and Grid Cells

The discovery of cells exhibiting striking spatial properties in the mEC supports the idea that this region of the brain provides a critical contribution to the hippocampal processing of spatial information: 'grid cells' (Hafting et al., 2005) display multiple discrete and regularly spaced firing fields that form a hexagonal grid pattern (Figure 2.4) that repeats over the whole 2D surface of a given environment. These grid cells are found close to the border between the mEC and the postrhinal cortex (Fyhn et al., 2004), in the dorsolateral band of the mEC (Hafting et al., 2005). Since their original discovery in the mEC, grid cells have also been reported in the pre- and parasubiculum (Boccara et al., 2010) as well as in other species such as mice (Fyhn et al., 2008), bats (Yartsev et al., 2011) and humans (Doeller et al., 2010).

2.4.1 General properties

The spatial firing of a grid cell is very regular and can be characterised by three main parameters (Figure 2.4B): the scale (size of and distance between neighbouring fields); the orientation of the grid (relative to an external reference); and the 'phase offset' of the grid (*i.e*. the displacement of the grid as a whole in the x,y axis, relative to an

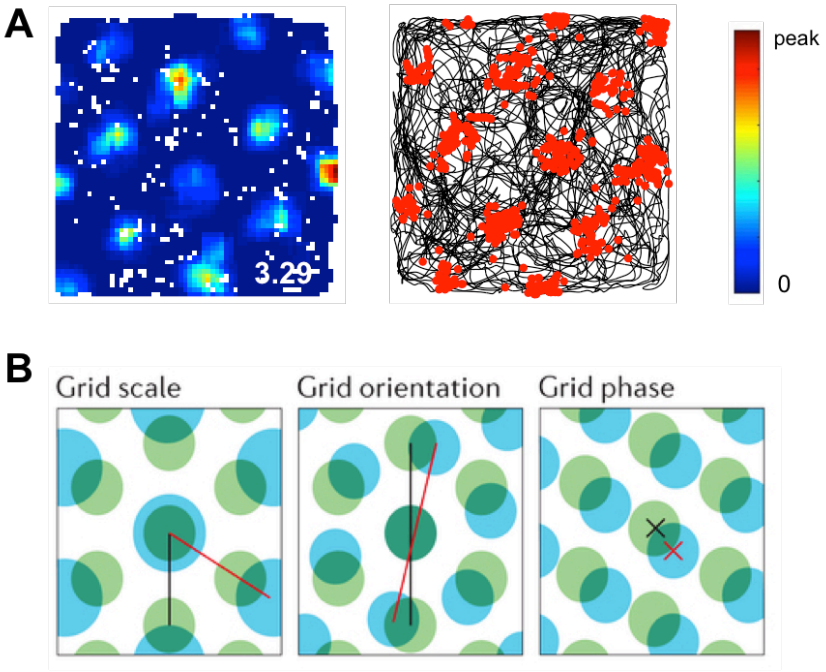


Figure 2.4: Grid cells and their properties. (A) The periodic firing of a grid cell can be seen in the rate map (right) and spike plot (left) of a single unit recorded from the mEC, in a 1.2m diameter circular environment (courtesy of G. Casali). In the spike plot, black lines indicate the path of the animal with the spiking events (red dots) superimposed on top. Peak firing rate (in Hz) noted at the bottom right of the rate map. (B) Salient properties of the grid cell firing pattern, with the fields from two different grid cells represented by green and blue circles [figure adapted from Moser et al., 2014]. Left panel: the scale (size or spacing of the grid) is the distance between two fields. Middle panel: the grid orientation is calculated as the angle between two fields from the vertical axis. Right panel: Grid phase, or displacement, is calculated as the distance from the centre of one field to the centre of the nearest field of another cell.

external reference). Simultaneously recorded grid cells appear to share the same orientation and scale (Sargolini et al., 2006) but there is variation in their phase offsets (Figure 2.4B). This variation in offset of neighbouring grid cells means that few grid cells are needed to accurately represent the whole 2D environment (Hafting et al., 2005). It has been suggested that grid cells are organised into discrete 'modules' with differences in orientation and scale (Stensola et al., 2012), exemplified by a linear increase in grid scale from ~25cm to ~3m over the dorsoventral axis of the mEC (Hafting et al., 2005; Brun et al., 2008). Interestingly, place cell field size mirrors the increase in grid scale: place field size also increases along the dorsoventral axis of the hippocampus (Jung et al., 1994; Kjelstrup et al., 2008), suggesting a strong relationship between grid and place cells.

2.4.2 Influence of sensory inputs on grid cells

The firing of grid cells, like place cells, is heavily spatially modulated. These cells generate their firing fields rapidly on exposure to a novel environment, and are able to retain spatially periodic firing regardless of changes to an animal's running speed or direction (Hafting et al., 2005; Fyhn et al., 2007). It has been postulated that grid cells (or at least a direct upstream input to them) receive both linear and angular self-motion information, and can process these together to extrapolate their current position with in an environment (O'Keefe and Burgess, 2005; McNaughton et al., 2006; Jeffery, 2007). Experimental evidence supports this idea as cells have also been found in the mEC that are modulated by head direction (Sargolini et al., 2006), as well as grid cells with a directional component (known as 'conjunctive cells'; Sargolini et al., 2006), cells that are modulated by speed (Kropff et al., 2015), and border cells (see section 2.7) (Solstad et al., 2008). Alongside these cell types, the mEC is part of an important information loop involving the hippocampal formation; the mEC therefore contains sufficient information about position, direction, distance, speed and boundaries in an environment (Sargolini et al., 2006) to construct an accurate representation of the animal's position in space.

Similar to place cells, grid cells are also affected by external cues. The rotation of prominent environmental landmarks causes grids to rotate by a corresponding angle (Hafting et al., 2005), though removal of landmarks in a familiar environment (or recording in darkness) does not impair grid cell firing (Hafting et al., 2005). Changes in environmental geometry have also been shown to affect scaling and spatial symmetry of grid cell firing (Krupic et al., 2015).

Taken together, these results indicate that grid cells may be important for encoding path integration and distance, and thus may provide the 'metric' component to the neural cognitive map proposed by O'Keefe and Nadel, 1978.

2.5 The Parahippocampal Region

2.5.1 Pre- and parasubiculum

At the border of the hippocampal formation and the parahippocampal region lie the pre- and parasubiculum. The relationship of these structures to the hippocampal processing loop can be considered as both inputs and outputs. Neurons in the pre- and parasubiculum project to layer III and II of the mEC respectively, stratum moleculare of the DG, subiculum, and the stratum lacunosum moleculare of CA3 and CA1. These structures also receive inputs from CA3, CA1 and the subiculum, as well as cortical projections from retrosplenial cortex and occiciptal visual cortex, and strong reciprocal connections with anterior thalamic nuclei (Witter & Amaral, 1995). The pre- and parasubiculum also contain spatially correlated cells (Boccara et al., 2010; Taube, 2007): grid cells (see section 2.3); head direction cells (see section 2.4); and border cells (see section 2.7).

The presubiculum is often divided, in the literature, into the presubiculum and dorsal presubiculum, also known as the postsubiculum. These structures are important to this thesis as they allow direct input to the HF from areas carrying the head direction signal: in addition to the aforementioned connection with the anterior thalamic nuclei (unique to the pre- and parasubiculum among the rest of the HF and parahippocampal region), the postsubiculum is also known to contain cells reflecting the current head direction of the animal (Taube, 2007). Cortical projections from the retrosplenial cortex also provide additional head direction input, alongside information from visual areas. Thus, it is likely that the head direction information is passed to hippocampal place cells via this route.

2.5.2 Perirhinal and postrhinal cortices

Both the perirhinal and postrhinal cortices receive many cortical inputs, and project onwards to the EC. PER is located dorsal to the EC and rostral to the POR in the parahippocampal region (Figure 2.1A), with the rhinal sulcus running along the majority of the rostrocaudal extent of PER. The two can be distinguished based on their connections with EC; PER predominantly projects to the lEC, and POR projects mainly to the mEC. This separation gives rise to a common proposition that there are two streams of information reaching the HF, each of PER-lEC and POR-mEC carrying different but complementary information.

PER gets input from many neocortical areas, receiving information from olfactory (*e.g.* piriform cortex), auditory, visual, visuospatial and sensorimotor regions (Burwell and Agster, 2008), while POR has only strong reciprocal connections with the visual and visuospatial regions (*e.g.* posterior parietal cortex, dorsal retrosplenial cortex, lateral posterior nucleus; Furtak et al., 2007) and other areas of the HF and PHR. Taken together, the diverse polymodal inputs to PER and POR, and experimental evidence, suggest that these regions are involved in encoding or processing contextual information about the environment (Burwell, 2004) which is then conveyed to the EC and HF. Experimental studies highlighting the importance of PER and POR to object recognition under changing context (Eacott and Norman, 2004) will be described in detail in Chapter 3.

2.6 The Head Direction System and Head Direction Cells

Fundamental to the positional coding of place cells and distance metric provided by grid cells, there are a set of cells dependent only on the orientation of the animal's head: head direction cells. These neurons were first noted by Ranck (1984), then confirmed in the dorsal presubiculum (known, as mentioned above, as 'postsubiculum') and characterized by his postdoctoral researcher Taube (Taube et al., 1990a, 1990b). Head direction (HD) cells fire maximally when an animal's head faces a particular direction in the azimuth ('horizontal') plane; this 'preferred firing direction' is independent of the animal's position or behaviour (Figure 2.5). A population of different HD cells have different firing directions, equally distributed and covering all 360° of the environment. This directional response is very stable, and neither the preferred firing direction (PFD), peak firing rate, nor directional firing range of a given cell in a familiar environment are seen to change over a period of weeks or months (Taube, 2007). The response, like place and grid cells, is also supramodal *i.e.* it is influenced by many sensory modalities (Blair et al., 1998; Knight et al., 2014). This point, alongside the properties of the HD system, will be expanded on in some depth in the subsections that follow in this thesis.

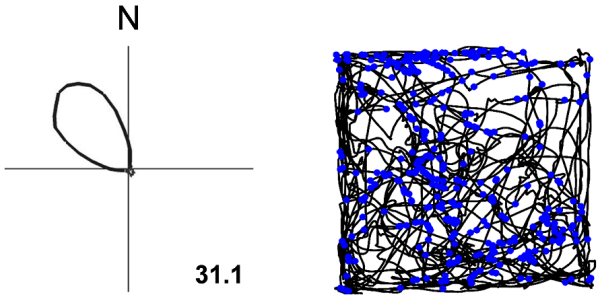


Figure 2.5: Example of a head direction cell recorded in the anterodorsal thalamus. This head direction cell, recorded by the author (D. Overington), can be represented by a polar plot showing a clear 'preferred firing direction' towards the North-West of the environment (left). The firing rate of the cell is noted in the bottom right of the polar plot, in Hz. Note that the spike map of a head direction cell shows no positional coding unlike place cells (right).

2.6.1 The Head Direction Cell Network

Since their discovery in the postsubiculum, HD cells have been found in several other areas of the brain including cortical regions: the retrosplenial cortex (Chen et al., 1994; Cho and Sharp, 2001), the medial entorhinal cortex (Sargolini et al., 2006), and the parasubiculum (Boccara et al., 2010); and subcortical regions: the lateral mammillary nucleus (Blair et al., 1998; Stackman and Taube, 1998), the dorsal tegmental nucleus of Gudden (Sharp et al., 2001), and the anterior thalamic nuclei (Taube, 1995; Tsanov et al., 2011). Areas with head direction cells are interconnected, providing a convergent signal from two information streams (Figure 2.6): a descending externally-derived (allothetic) stream providing static sensory information from cortical areas; and an ascending internally-derived (idiothetic) stream providing dynamic movement-related information from thalamic vestibular circuits (Bassett and Taube, 2005; Taube, 2007). Through this densely interconnected network, directional information then ends up in the hippocampus via the parahippocampal region and entorhinal cortex.

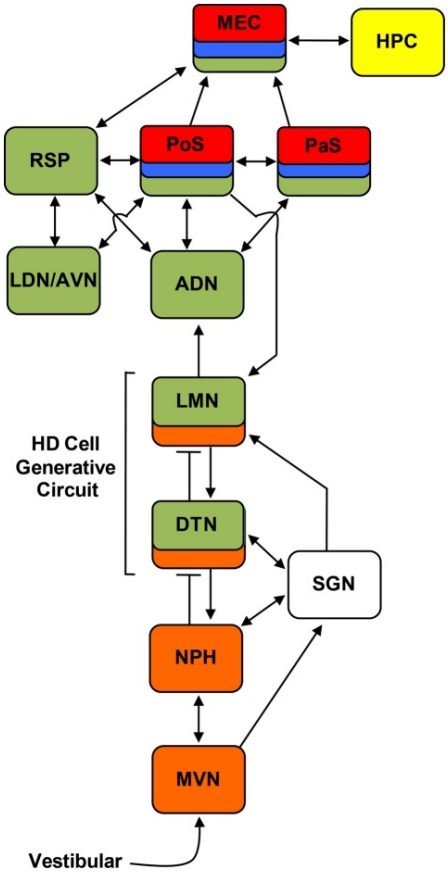


Figure 2.6: Circuit diagram showing principle connections between brain regions in the head direction network. Colours signify existence of particular cells: yellow (place cells); red (grid cells); blue (border cells); green (HD cells); orange (angular velocity cells). Vestibular information enters the dorsal tegmental nucleus (DTN) from the nucleus hypoglossi (NPH) and medial vestibular nucleus (MVN). From there, information flows up to the lateral mamilliary nucleus (LMN) and anterodorsal thalamic nucleus (ADN), and gets passed on to the medial entorhinal cortex (mEC) and lateral dorsal nucleus (LDN) via the retrosplenial cortex (RSP), postsubiculum (PoS) and parasubiculum (PaS) before entering the hippocampus (HPC). Visual input from visual cortex (not in diagram) enters the network through RSP and PoS. Arrows with arrowheads represent excitatory projections; arrows with flat heads represent inhibitory projections. Figure from (Clark and Taube, 2012).

It is unclear why the directional signal is replicated across many different brain regions, though none of these areas can be considered strictly HD cell areas as the proportion of cells displaying directional firing characteristics varies and is never 100% (Taube, 2007). For example, in the anterodorsal thalamic nucleus (ADN) approximately 60% of neurons can be classified as HD cells (Taube, 1995), while this figure is closer to 25% in the postsubiculum (PoS) (Taube, 2007) and lateral mammillary nucleus (LMN) (Stackman and Taube, 1998). Cell firing

properties and peak firing rates are broadly similar across regions, but there are variations in average tuning curve width with cells showing firing over more or less than the average 90°.

2.6.1.1 Lesion studies indicate a roughly hierarchical organisation of head direction areas

Lesion studies revealed that disruption of the ADN abolishes HD cell firing in the PoS (Blair et al., 1999). Conversely, Goodridge & Taube (1997) found that lesions to PoS have no effect on directional firing in the ADN. PoS lesions do, however, increase the directional tuning curve width of ADN HD cells, demonstrating a reduction in accuracy of the ADN HD signal (Goodridge and Taube, 1997). Other studies have also found a lack of disruption to the ADN head direction signal following lesions of RSC (Clark et al., 2010) and mEC (Clark and Taube, 2011). Similarly, ADN lesions also do not affect place cell activity in the HPC, but disruption of the PoS causes place field instability (Calton et al., 2003); this suggests that a lack of visual input via the PoS to HPC (through EC) causes place fields to be more reliant on self-motion path integration information and thus subject to increased error and instability.

ADN HD cell firing is, however, abolished by lesions of the DTN (Bassett et al., 2007) or LMN (Blair et al., 1999). Indeed, LMN lesions also seem to abolish firing in PoS (Sharp and Koester, 2008); this is unsurprising given the already established dependence of the PoS on an intact ADN HD signal.

Overall, these lesion studies reflect a roughly sequential flow of the HD signal in a central circuit from the DTN/LMN to ADN to PoS and other parahippocampal structures (Figure 2.6), with the DTN/LMN appearing to be the site of HD signal generation. The two streams of information (descending sensory and ascending self-motion information) are thought to come together at the ADN, which then would act to process and integrate the inputs for the HD system (Taube, 2007).

2.6.2 Allothetic inputs to the HD system

One determinant of HD cell firing comes from sensory information available in the external world, including visual, olfactory, auditory and tactile information. These allothetic inputs are obvious candidates to anchor the animal's orientation within a given environment, and seem to influence HD cell activity in a varied manner, as described below.

2.6.2.1 Visual information

In the initial body of published HD cell work, Taube et al., (1990b) investigated the effects of environmental manipulations on the firing properties of HD cells. First, the importance of visual landmarks was assessed using a cue-rotation paradigm: a cue card covering a 100° arc of the cylindrical environment was rotated either 90, 180 or 270° between recording sessions, while the animal was also mildly disoriented between sessions. After each rotation of the cue card, HD cells were shown to rotate their preferred firing direction (PFD) by the same amount *i.e.* after a 90° cue card rotation, the PFDs of simultaneously recorded HD cells also rotated by around 90°. However, it should be noted that HD cells tend to slightly under-rotate with respect to the cue card rotation (Knight et al., 2014; Taube, 1995; Taube et al., 1990b). Despite this, results of cue-rotation experiments show that visual cue cards are used by the HD system to stably anchor the PFD of cells to the external environment. This 'landmark control' of HD cell PFDs has been shown to hold true throughout the HD cell circuit (ADN: (Taube, 1995); RSC: Chen et al., 1994; LMN: Stackman and Taube, 1998; MEC: Sargolini et al., 2006). Similarly, place cells recorded under a cue-rotation paradigm also rotate accordingly with the cue rotation (Muller and Kubie, 1987).

Taube et al. (1990b) also showed that even though visual landmarks drive the PFD of HD cells, they are not necessary for the maintenance of a stable directional signal. In a cue card removal paradigm, the authors showed that HD cells maintained directional modulation (with similar peak firing rates and cell tuning widths) comparable to sessions when the cue card was present. Thus, HD cells are able to function based on path integration alone but in these conditions tend to drift (noted by a shift in the PFD between cue present and cue removal sessions); this drift is likely to be driven by error accumulation from idiothetic signals resulting in under- or over-estimation of the degree of cue rotation. Similar results were obtained when HD cells were recorded in the ADN and PoS from blindfolded rats (Goodridge et al., 1998); HD cells maintained strong directional modulation without the presence of visual cues, with comparable firing rates and tuning widths in both standard and blindfolded sessions. Again, there was an average PFD shift of 23° during the blindfolded sessions (considerably larger than the ~6° shift of standard sessions); this supports the notion that HD cells do not require visual information for the directional signal, but that they need to be updated by landmark information to anchor their PFDs.

The experiments discussed above established that visual landmarks are important for maintenance of stable PFDs, though in natural navigation landmarks tend to have different characteristics and are not all as salient as a large white cue card. Some

natural landmarks will be in the background and seen from all angles, some may be more familiar, and others may not always be present. To assess whether properties of landmarks have an effect on landmark control, HD cells were recorded from the ADN in environments with either only proximal cues (*i.e.* foreground objects) or both proximal and distal cues (*i.e.* objects plus distal cue cards; Zugaro et al., 2001). In this study, three objects were placed at equal distances at the periphery of a circular platform; this platform was either enclosed within a blank-walled cylinder (proximal condition) or left open so the animals could view a distal curtained backdrop (distal condition). In each condition, animals were tested in a baseline session and 120° object rotation session. When PFDs were compared between the baseline and rotation sessions for each condition, the authors found that in the proximal condition HD cell PFDs rotated accordingly with the objects but that there was no PFD rotation in the distal condition. These findings suggest that distal cues are favoured as anchoring points for HD cell firing over proximal cues, reflecting a possibility that distal cues are usually treated as more stable and reliable.

2.6.2.2 Olfactory and auditory inputs

In addition to studying the role of visual landmarks on HD cell firing, Goodridge et al. (1998) also manipulated olfactory and auditory cues. In their olfactory cue study, four cotton buds were taped at equal distances around the edge of a cylindrical apparatus. Of the four, three were left odourless while the fourth was dipped in peppermint extract. The experiment compared firing characteristics of HD cells between a baseline session and a rotation session where the cotton buds were rotated by 90°. Similarly to the visual landmark rotation experiments, the majority of HD cells rotated in the same direction as the olfactory cue but there was a large degree of under-rotation, and the PFDs did not follow a return rotation to baseline. Compared to a 16° average in visual landmark rotation experiments, PFDs in the olfactory cue rotation experiment under-rotated by ~55° and deviated from baseline by ~89° on return rotation. Together, these results show that while HD cells can use olfactory cues to orient their directional firing, there is a hierarchical preference for using visual landmark information. The experiments in this thesis rely on odour cues to disambiguate visually identical but oppositely oriented spaces; maintenance of a globally consistent PFD would require the HD cell to integrate the location of the visual cue within the odour context, and if only visual cues were relied upon then we would expect the PFD to rotate 180° between compartments. This will be expanded upon further in Chapter 5.

A similar approach was taken to determine the salience of auditory cues in anchoring HD cell firing: auditory stimuli were presented to animals from a particular direction

during baseline sessions, and from a direction 90° clockwise to this in the rotation condition (Goodridge et al., 1998). Unexpectedly, HD cells showed no tendency for their PFDs to follow the auditory click rotation, with an average deviation of 112° from their expected firing direction. Thus these results indicate that, unlike visual landmark and olfactory cues, auditory information is not used as an orienting cue by the HD system.

2.6.2.3 Summary

Studies into the allothetic inputs for the HD system show a hierarchical preference for visual cues, similarly to place and grid cells, in the anchoring of HD cell firing activity. Olfactory cues exert some control but play a minor role when stable visual landmarks are available, while auditory cues do not seem to provide any source of orientation under any condition. Though visual and olfactory cues seem to anchor the directionality of the HD system, removal of salient allothetic cues does not result in complete degradation of directional firing. This indicates that the HD signal can be generated purely by internal information, but relies on allothetic information for accurate updating of direction with relation to the external world.

2.6.3 Idiothetic inputs to the HD system

Without the presence of external landmark information, it is still possible for animals to navigate accurately using a process known as 'path integration'. This type of navigation is thought to rely solely on the presence of internally generated, or 'idiothetic', signals (Mittelstaedt and Mittelstaedt, 1980). As mentioned above, the HD signal is present even without external landmark cues (*e.g.* in cue-removal experiments, Taube et al., 1990b, or blindfolded rats, Goodridge et al., 1998), indicating that the HD system can maintain directional firing using idiothetic cues alone. Types of idiothetic cues include vestibular information, motor efference copy, proprioception and optic/auditory flow; the role of these cues in the generation and maintenance of the HD signal will be discussed in the sections that follow.

2.6.3.1 Vestibular input

The primary sources of vestibular input to the HD system come from the vestibular end organs (Stackman and Taube, 1997; Stackman et al., 2002; Yoder et al., 2011). This structure consists of three differently oriented semicircular canals and two otolith organs, which are responsive to rotations of the head (pitch and roll) and linear head acceleration respectively. The modulation of the horizontal canals by yaw (horizontal) rotations is what makes them the ideal candidate for influencing, or indeed generating, the HD signal.

The strongest evidence that the vestibular signal is essential to the generation of HD activity came from studies that disrupted activity of the vestibular end organs (both permanently and reversibly) and aimed to record from the HD system (Stackman and Taube, 1997; Stackman et al., 2002). In the earlier study, lesions of the vestibular apparatus eradicated directional tuning in the ADN (Stackman and Taube, 1997): when lesions were performed before looking for cells, no directional activity was found; and when lesions were performed after positive isolation of HD cells, the directional tuning was eradicated within 96 hours post lesion. Temporary lesions of the vestibular organs using tetrodotoxin yielded a similar effect (Stackman et al., 2002): there was a temporary loss of directional tuning in PoS HD cells, and a coincident loss of place cell location-specific firing. These experiments reveal that the vestibular end organs, and perhaps the vestibular signal as a whole, are necessary for the generation of the directional signal in both the ADN and PoS. Importantly for this thesis, they also indicate that loss of directional modulation in these areas is correlated with a loss of location-specific place cell activity.

2.6.3.2 Motor cues and optic flow

For a behaving rat, the motor cues provided to the HD system may be in the form of motor efference copy, motor command signals, and proprioceptive (joint movement and position) signals. Evidence for the role of motor cues in generating the HD signal comes from experiments where rats were passively rotated whilst restrained or immobile; under these conditions, passive rotation caused a significant reduction in peak firing rate of HD cells in the PoS (Taube et al., 1990b) and ADN (Taube, 1995) but did not affect their directional tuning. In addition to this, it was found that passive transport of rats between two areas in both light and dark conditions resulted in a robust shift in PFD similar to when the rats actively transported between the two (Stackman et al., 2003). Together, these results indicate that motor signals in active behaviour are important for maintaining stable directional tuning while also potentially influencing the firing rate of HD cells.

More recently, Shinder & Taube (2011) recorded ADN HD cells while rats were both restrained and head-fixed to a rotating apparatus, in both active and passive transport conditions. In contrast to the studies discussed above, firing rates and PFDs of HD cells during passive rotation were comparable to those of active transport. The authors also reported that passive rotation results in darkness showed no obvious differences in firing rates and directions, only an increased tuning width. This suggests that the vestibular system is sufficient for generating accurate directional representations in HD

cells, and that the influence of motor cues may be inconsequential in maintaining a stable directional signal.

The studies of Stackman et al. (2003) and Shinder & Taube (2011) also investigated whether optic flow was important to the HD signal. Optic flow results from the relative motion of the observer *i.e.* the animal, and the world around them; movement of the head or eye results in a rotation of the visual field on the retinae, which can then provide information about the head motion of the observer. The two experiments demonstrated that there is also a hierarchy in the importance of idiothetic inputs to the HD system such that optic flow does not strongly affect the HD signal when either vestibular or motor cues are present. This comes from the findings that there are no significant changes compared to baseline HD cell firing properties even when the animal was passively transported in darkness (*i.e.* when motor and optic flow cues were removed; Shinder and Taube, 2011; Stackman et al., 2003).

2.6.4 Summary of inputs to the HD system

Overall, it is clear that while allothetic cues are necessary for anchoring HD cell firing to external landmarks, idiothetic signals generated by the vestibular system are first necessary for the generation of the HD signal (Stackman and Taube, 1997; Stackman et al., 2002). This idea is supported by studies of the HD system in development: the HD signal is present before eye opening in P12 rat pups (*i.e.* before visual information is available) but at this point lacks directional information content and stability; however, within 24 hours of eye opening, the system then matures very quickly to a point where visual landmark information is able to control HD cell responses in cue-rotation experiments (Tan et al., 2015). Thus, similarly to place cells, both allothetic and idiothetic cues are required for the HD system to generate and maintain stable directional firing that updates relative to the external world.

2.7 Boundary Vector Cells and Border Cells

Early experimental observations showed that hippocampal place cell firing often seemed to be determined by geometric constraints of an environment (see Chapter 3) (O'Keefe and Burgess, 1996). This led researchers to propose that place cells receive inputs from cells called 'boundary vector cells', a set of neurons that fire at a given distance/allocentric direction from a boundary (Hartley et al., 2000). The boundary vector cell (BVC) model of place cell firing (Barry et al., 2006; Burgess et al., 2000; Hartley et al., 2000) postulates that the convergence of inputs from a population of

these BVCs, tuned to all the possible boundaries in an environment, could result in the more localised firing seen in place cells.

Cells that resemble the computational modelling of BVCs have been found in the subiculum (Barry et al., 2006; Lever et al., 2009), presubiculum, parasubiculum (Boccara et al., 2010), and medial EC (Solstad et al., 2008; Bjerknes et al., 2014). In line with the model, these neurons have firing fields that increase in rate the closer the animal is to a given boundary (Figure 2.7) and can also fire along perpendicular borders (e.g. around a corner). These neurons can also follow the shape of boundaries such that in a square/rectangular environment their fields are linear but in a circular environment the field will be curved, and develop additional fields if another barrier is introduced (Lever et al., 2009). The definition of 'boundary' in the case of these neurons is somewhat undefined at this time, as objects positioned in a row or drops off the edges of a raised arena (Lever et al., 2009; Stewart et al., 2014) can also elicit BVC firing. Unlike other spatial cells, BVCs seem not to be influenced by non-metric cues like colour, odour or texture (Lever et al., 2009) and are mainly driven by memory of the boundary's position relative to the animal from path integration and optic flow information (Raudies and Hasselmo, 2012). Given that the areas with these BVCs are all connected reciprocally (directly or indirectly) with the hippocampus, the BVC model of place cell firing mentioned above may be plausible. Supporting this, it was found experimentally that if boundaries were removed from the recording arena then place field activity would break down (Barry et al., 2006; O'Keefe and Burgess, 1996), so these geometric features and the neurons encoding them within the brain definitely play an important role in the animal's representation of space.

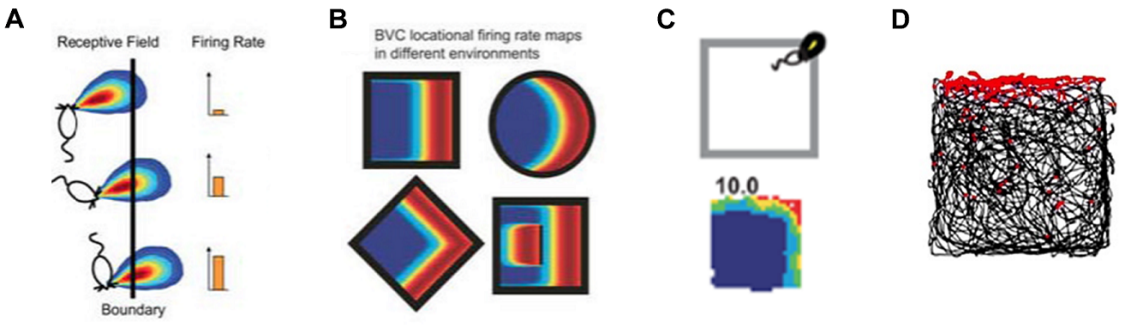


Figure 2.7: Cells responding to environmental boundaries – boundary vector cells and border cells. (A,B) The boundary vector cell model of response to boundaries: as the animal moves closer to a given boundary, the firing rate increases until maximal at the boundary itself. Shown in B, the firing of BVCs is consistent over different environments and can span corners and exhibit extra fields if an additional boundary is introduced. (C) Response of a single BVC recorded in the postsubiculum, if the animal is at the location indicated by the top panel. The peak firing rate of the cell is above the rate map, in Hz. Panels A, B, and C are adapted from Lever et al., 2009. (D) Example spike plot of a border cell recorded in the entorhinal cortex [taken from Marozzi and Jeffery, 2012], note that border cells seem to only fire immediately next to a boundary.

Another class of cells that fire along boundaries are termed ‘border cells’, and are found in all layers of the EC (Solstad et al., 2008). The firing of border cells, like BVCs, is stable with regard to environmental change, suggesting that they too are purely defined by geometric borders and the animal’s internally generated information (Solstad et al., 2008). However, in contrast to BVCs’ graded firing in intensity with distance from a boundary, border cells only seem to fire closely along walls of an environment (Solstad et al., 2008). This distinction suggests that BVCs and border cells are distinct cell types, though it is also possible that border cells are just a highly tuned set of BVCs (tuned to fire at very short distances to boundaries; Lever et al., 2009).

2.8 Summary

Overall, this chapter has shown that a complete spatial map indeed requires all the elements posited by O’Keefe & Nadel (1978) and that this information is well represented within the rodent brain (among other species): a taxon system of navigation requires a representation of distance, direction and position, which can be respectively provided by grid, HD and place cells. Place cells in the hippocampus signal an animal’s location within a particular environment (O’Keefe and Nadel, 1978) while one synapse upstream in the mEC, grid cells combine angular and linear self-motion to produce a metric signal (seen as a periodic hexagonal firing over the environment) that can keep track of how far an animal has moved through space (Hafting et al., 2005). Head direction cells then provide the ‘compass’ of the brain’s navigational system, reporting the direction of the animal’s head in relation to allocentric space (Taube, 2007). Other spatial cell types including border cells, boundary vector cells, grid-by-head direction and grid-by-direction-by-speed cells have also been reported throughout the HF and PHR, providing complementary information to the network about an animal’s environment. In current research, the strict hippocampus-centered cognitive map proposed by O’Keefe and Nadel (1978) has been expanded upon to include facets of the one put forward by Tolman (1948): the brain’s spatial system must contain spatial, contextual and non-spatial information, and appears to be spread over multiple brain regions (with the HPC as its likely centre). Future chapters of this thesis will explore how contextual information can inform the spatial network about different environments and how neurons, and animals, tackle being presented with visually identical but contextually different spaces.

Chapter 3 - The Cognitive Map and Spatial Ambiguity

As noted in Chapter 2, visual cues in the environment act as salient cues for hippocampal place cells, grid cells, head direction cells and boundary cells. In the presence of stable visual cues in an environment, place fields can present with consistent fields for weeks or even months (Muller et al., 1987; Thompson and Best, 1990). Rotation of visual landmarks causes notably causes place fields (Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Shapiro et al., 1997), and head direction cell preferred firing directions (Skaggs et al., 1995; Yoganarasimha et al., 2006), to rotate with the stimuli. Given the level of control exerted on spatially tuned cells by visual cues, how do these cells reconcile the ambiguity in environments that look visually similar? Can spatial cells discriminate two distinct but visually identical environments?

3.1 How can we tell different spaces apart?

Human episodic memory allows for the association of "what", "when", and "where" aspects of an event all coded in relation to each other, and voluntary recall of episodic memories often involves first thinking of the location of the 'episode' as a trigger to recall the rest of events. This suggests that the location *where* something happened is very important to the events of *what* happened, and that the two together are needed for proper recall (Tulving, 1983). Place cells can be considered the positional output of "where" in spatial representation, but there is also much in the background of where events occur that could provide key information to distinguish one visually similar environment from another.

The background cues in an animal's environment, complementary to salient visual cues, are often referred to as the 'context' (Mizumori et al., 1999); here, the 'spatial context' will refer to the combination of geometric (comprising the information about geometric features e.g. shape and orientation) and contextual (non-metric information e.g. colour[1] and odour) information. Together, visual cues and spatial context should be able to tell the animal which environment it is in and where it is within it; and thus spatial context should also be able to provide a way for the animal to solve visually ambiguous space.

3.1.1 Context and Objects

Researchers have used object recognition as a means to explore how context can be used to inform an environment, termed 'object-in-context'. Tasks involving object

[1] It is important to note that rats have monochromatic vision, so 'colour' in this thesis refers to the brightness/luminance of a given stimulus.

recognition in context are based upon the fundamental principle of a novelty-preference memory paradigm (or 'spontaneous recognition task'), exploiting rats' spontaneous exploratory behaviour and their innate preference to investigate instances of change (novelty) in a familiar environment. A 'non-matching' condition is experimentally created between the learning phase and the memory test, such that the animal will express its memory of the original learning experience by preferentially exploring the novel stimulus over familiar ones (Ennaceur and Delacour, 1988).

In the simplest version of an 'object-in-context' paradigm (the task used in Chapters 5 and 7 are a variant of this), rats are exposed to two identical objects under one set of contextual cues (e.g. an environment with white textured walls) and two other identical objects in a second context (e.g. an environment with black untextured walls). In the test phase, rats are presented with two familiar objects, only one of which had not been encountered in the current location and context. From fundamental principles, the prediction stands that if the animal was able to learn about the object as well as the background cues of the spatial context, they should spend more time investigating the object that is novel to that particular context. This hypothesis was true for rats encountering new objects under standard conditions (Eacott and Norman, 2004), suggesting that rat behaviour is in fact influenced by the spatial context of the environment.

The hippocampus is widely thought to play an important role in the memory of events and the environment/place in which they occur (Kim and Fanselow, 1992; O'Keefe and Nadel, 1978). Similarly, rodent studies using variants of the spontaneous recognition task (as detailed above) lend support for the view that context-dependent association requires the hippocampus. Rats with hippocampal lesions typically could not perform the previously described recognition task to above-chance levels, such that any objects encountered by lesioned animals seemed to be encoded equally without reference to a particular context (Good et al., 2007; Mumby et al., 2002; Norman and Eacott, 2005; Save et al., 1992). These impairments were consistent when the object recognition was tested with spatial location (Langston and Wood, 2010; Mumby et al., 2002; Good et al., 2007), background context (Mumby et al., 2002) or integrated recognition of objects, location and recency of presentation (Good et al., 2007) or integrated recognition of object, location and context (Eacott and Norman, 2004; Langston and Wood, 2010). However, hippocampal lesioned animals showed no impairment in the recognition of individual objects themselves (Ainge et al., 2006; Mumby et al., 2002; Good et al, 2007; Langston and Wood, 2010), indicating that context-independent object recognition can be supported by processes independent of the hippocampus.

Researchers have also investigated whether the impairments seen with hippocampal lesions would be replicated with lesions to areas known to be connected to the hippocampus. Rats with fornix lesions were impaired on a task requiring complex recognition of object, location and context, but unimpaired on an object-place version of the same task (Eacott and Norman, 2004). Interestingly, when fornix lesioned animals were tested on an object-context version of the task, they were only impaired when there was a long delay between presentation and recall (Norman and Eacott, 2006). These findings, supported by the results of Langston and Wood (2010), are interesting because they suggest that associative recognition including context is not always hippocampus dependent, and that processes underlying object-place context recognition are potentially not the same as those dealing with its separate parts (object-place and object-context recognition).

When looking at the effect of the parahippocampal region in such tasks, it was found that rats with lesions to the PER and POR were differentially affected in situations involving context-driven object recognition: at short delays post familiarisation, PER lesioned rats were unimpaired in context object memory while those with POR lesions were severely impaired (Norman and Eacott, 2005). Conversely, rats lesioned in the PER were impaired in a non-contextual object recognition task while hippocampal and POR lesioned rats were unimpaired (Norman and Eacott, 2005). Taken together, these results suggest that PER is important for object recognition, but POR is important for associations between spatial context and objects. This also lends support to the dual systems theory of context representation (Rudy, 2009) that suggests there are two neural systems processing contextual information: one of which includes the hippocampus, and another that instead involves the parahippocampal network and neocortex.

3.2 How do neurons represent different spaces?

How might neurons represent different environments? It will be argued throughout this thesis that cells of the hippocampal place system (in particular, place and head direction cells) are capable of discriminating even visually similar connected spaces with differing spatial context as separate environments.

It is now well established that hippocampal place cells respond not only to geometric aspects of an environment (*e.g.* walls or boundaries; Muller and Kubie, 1987; O'Keefe and Burgess, 1996) but also to more the complex cues that make up spatial context (Anderson and Jeffery, 2003; Anderson et al., 2006). These contextual cues, as previously mentioned, encompass a wide variety of non-metric information about the

environment such as colour and odour (Anderson and Jeffery, 2003; Bostock et al., 1991), the route the animal has to take within an environment (Frank et al., 2000) or even the internal expectation of the animal about which turn to make on a track (Ainge et al., 2007; Wood et al., 2000).

3.2.1 Place cell remapping

Place cells appear to be able to distinguish between different environments, or react to a change made in a familiar environment, through non-parametric changes to their place field firing. This phenomenon is known as 'remapping' (Muller and Kubie, 1987; Bostock et al., 1991) and can manifest in three main ways (Figure 3.1).

- **Complete or global remapping:** when all place cells recorded change their activity simultaneously, but not always in the same manner. Some place cells will stop firing, while others begin to fire or alter the location of their place fields (Figure 3.1C).
- **Partial remapping:** when some place cells in a population alter their firing, but other co-recorded cells remain stable (Figure 3.1D).
- **Rate remapping:** when place field location remains stable but the rate at which place cells fire within the field either increases or decreases substantially (Figure 3.1E).

Muller and Kubie (1987) found when rats were familiarised with a circular environment and subsequently placed in a novel rectangular environment, all co-recorded place fields completely remapped (as the description above). However, when rats went from the same familiar circular environment to a novel larger circular environment, only some place cells in an ensemble remapped while others remained stable *i.e*. only partial remapping occurred. Although complete remapping is more commonly reported in the literature, there is more and more evidence that that place cell ensembles often do not remap consistently but in fact frequently do display partial remapping in response to environmental changes (Skaggs and Mcnaughton, 1998; Lever et al., 2002; Anderson and Jeffery, 2003; Moita et al., 2004). For example, partial remapping was found in response to a contextual fear conditioning paradigm where some cells in a given ensemble remapped post conditioning and others remained stable (Moita et al., 2004). In addition to this, minor changes to an environment like inserting a barrier in a maze to block a previously available route, or conversely removing a barrier to create a shortcut, also can induce partial remapping in place cells (Alvernhe et al., 2008, 2011): in these studies, it was found that CA1 and CA3 place cells with fields close to the site of environmental manipulation were more likely to demonstrate partial remapping than

those with fields further from the site of change. Similarly, when objects within an environment are rotated then cells with place fields closer to the objects would be more likely remap (with ceased firing or change in place field location) than those with place fields further from the objects (Lenck-Santini et al., 2005). These types of examples (Alvernhe et al., 2008; Alverhne et al., 2011; Lenck-Santini et al., 2005) are more specifically referred to as 'local remapping', where the partial remapping is localised to place fields in proximity of the manipulation.

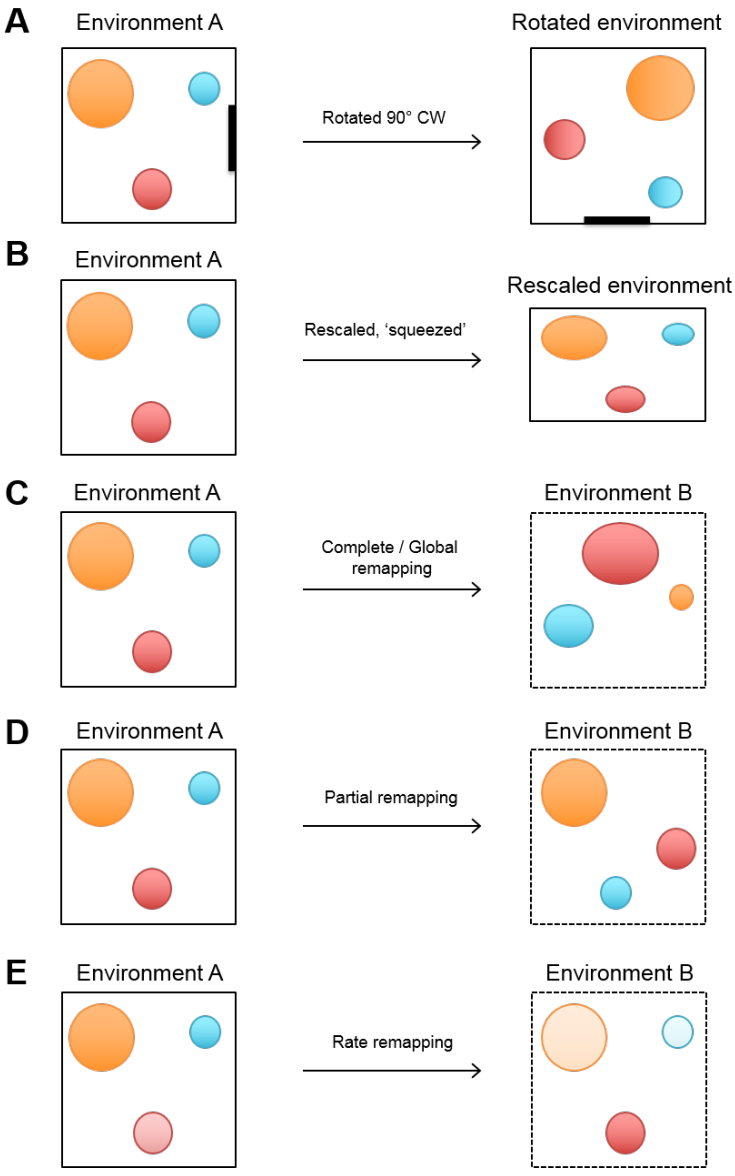


Figure 3.1: Summary of place cell responses to environmental manipulations. Each coloured oval represents the activity of one place cell. (A) Place fields follow rotation of an environment, if there is a salient distal cue present (here, a cue card on the wall). (B) Parametric rescaling of place fields occurs following environmental rescaling. (C) Complete remapping results in all recorded place cells altering their firing by either changing location of their place fields or by ceasing or starting to fire. (D) Partial remapping results in some place cells firing in a different location or ceasing/starting to fire, while others remain stable. (E) Rate remapping results in differential firing rates of place fields between environments without any change in field location.

The third type of remapping, termed 'rate remapping', presents as a difference in firing rate between two separate environments while the place field locations remain the same (Hayman et al., 2003; Leutgeb et al., 2005). Although rate remapping is sometimes less pronounced than remapping events involving field location changes, there are events where rate remapping can result in firing differences within the hippocampal population with the same amount of dissimilarity as observed with global remapping *e.g.* in one environment the cell will fire at a peak rate of less than 1 Hz within its given field, but in another the rate in the same field (from the same cell) can be up to tenfold higher (Hayman et al., 2003; Leutgeb et al., 2005). However, rate remapping is sometimes hard to distinguish from global remapping as a substantially decreased rate in one field of a place cell alongside an increased rate in another field could appear as a shift in firing location.

The phenomenon of remapping, and the distinction between global and partial remapping, may therefore help the spatial system to distinguish between similar but different environments.

3.2.2 Place cell remapping and spatial context

Partial remapping has also been shown in response to changes to the colour and odour of an environment *i.e.* the spatial context (Anderson and Jeffery, 2003). The experiment of Anderson and Jeffery (2003) is important to examine as similar changes to the spatial context, specifically odour, were made in the experimental chapters of this thesis.

Place cells were recorded in four compound contexts (black-lemon, black-vanilla, white-lemon, white-vanilla), while the geometry and position of the environment within the recording room remained the same. It was found that purely manipulating the combinations of contexts elicited heterogeneous partial remapping responses in place cells (Anderson & Jeffery, 2003): for example, cell 1 fired in only the lemon scented boxes but at different locations depending on the colour (i.e. fired differentially in black-lemon and white-lemon, but not at all in black-vanilla or white-vanilla), while cell 2 fired in the same manner in black-lemon, white-lemon and black-vanilla but not at all in white-vanilla (Figure 3.2). In addition to confirming that changes to the environment deemed 'less complete' will still elicit remapping and the distinction between environments (global remapping seemingly requires 'large scale' changes such as altering the recording room or all the distal cues), this study also provided insight into how the hippocampus represents and processes contextual information. Firstly, the heterogeneity in partial remapping suggests that not all place cells are receiving the

same information about context given that co-recorded place cells respond differently to different combinations of contextual cues. Secondly, the site where contextual information is combined must be up- or downstream of the CA1 place cell network, potentially in the dentate gyrus (Hayman & Jeffery, 2008) or other parahippocampal and connected cortices.

3.2.3 Place cells and environmental rescaling

Geometric boundaries also have influence on place cell representations: radical geometric changes will induce remapping (such as a square directly changing to a circle), but more subtle changes can influence place fields differently through a process termed 'environmental rescaling'. Early studies reported that increasing the size of a circular recording arena could result in place field expansion or 'rescaling' (Muller and Kubie, 1987), such that the amount of field expansion somewhat correlated with the expansion of the arena. The phenomenon of field rescaling was later confirmed by O'Keefe & Burgess (1996), finding that if a square environment was 'squashed' to a rectangle in one dimension (the same walls were used in each case, and the centre of the environment remained constant within the room) then place fields immediately underwent parametric rescaling in line with the new shape of the environment (Figure 3.1B). The location of the cells' firing field(s) and peak firing rate remained consistent relative to the walls of the environment, but some place fields became elongated along the altered axis of the environment, and in the largest environments some fields

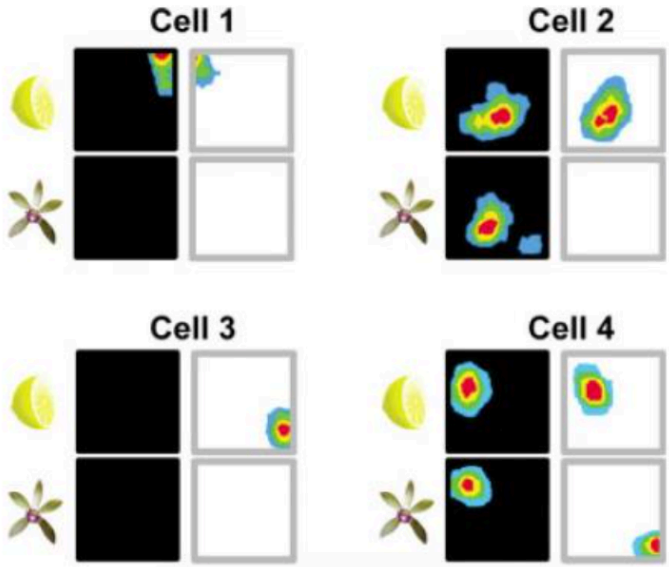


Figure 3.2: Example of place cell partial remapping in response to contextual changes. Changes to colour (black and white) and odour (lemon and vanilla) of an environment can illicit different partial remapping responses from co-recorded place cells; this suggests that neighbouring cells do not receive entirely the same information about the spatial context of an environment but that they can tell similar environments apart. For example, cell 3 only fired in the white-lemon condition, while cell 1 fired both (but with dissimilar field locations) in the black-lemon and white-lemon conditions. Figure from Anderson & Jeffery, 2003.

became bimodal (O'Keefe and Burgess, 1996). In line with this, when a familiar environment was reduced in size then the place fields tended to rescale along the axis of the deformation. These experiments give clear evidence that place fields are sensitive to external environmental cues and can flexibly update their representation in response to changes in these cues.

3.2.4 Grid cell remapping

Similarly to place cells, grid cells also remap in response to environmental changes, but the manner of remapping is different. Large changes to the environment that would cause global remapping of place cells will cause translation and/or rotation of grid cell firing fields (Fyhn et al., 2004; Marozzi et al., 2015).

mEC grid cells were co-recorded with CA3 place cells under conditions which are known to induce either complete or rate remapping in place cells (Fyhn et al., 2007). It was found that instances of rate remapping in CA3 place cells (by manipulating the colours of the walls) were associated with stable grid fields that maintained a consistent rate, offset, orientation and scale in both environments. Conditions that result in partial remapping of place cells (by manipulating combinations of colour and odour), resulted in purely translational realignment in grid cell firing patterns with no observation of rotation (Marozzi et al., 2015). However, instances of complete remapping in CA3 place cells (by changing the environment from a square to a circle or using different recording rooms) were always accompanied by a coordinate shift in the offset and/or rotation of all co-recorded grid cells i.e. the grid cells 'realign' to the new environment (Fyhn et al., 2007). Together, these findings suggest that grid cells are more robust to smaller changes in the environment, and even when large changes influence their firing then their intrinsic spatial firing pattern (the spacing and scale of fields) is retained.

3.3 How do neurons respond to visually similar spaces?

While visual landmark cues play an important role in updating the spatial firing of place cells, it is now understood that these neurons are influenced by a combination of multiple sensory modalities that are both external and self-generated. This understanding first came from experiments that recorded relatively stable place cells in complete darkness (Save et al., 2000; Zhang et al., 2014) and equally relatively stable place cells in rats blinded from an early age (Save et al., 1998). In these cases, it was proposed that one of the most important drivers of place cell firing was path integration (PI) (Etienne and Jeffery, 2004; Mittelstaedt and Mittelstaedt, 1980); an internally generated and updated representation of position based on vestibular and proprioceptive information from an animal's movement over time. Many experiments

have confirmed that place cell firing is, at least to some extent, influenced by PI information (Etienne and Jeffery, 2004; Gothard et al., 1996) and have gone on to ask whether this internally generated information is enough to separate visually identical but spatially separate environments. On recording place cells in two identical compartments joined by a corridor, it was interestingly found that the hippocampus did not appear to consistently form orthogonal (statistically unrelated) representations for different environments and also did not seem to form a consistent representation within an environment (Skaggs and McNaughton, 1998; Fuhs et al., 2005). Skaggs and McNaughton (1998) found that many place cells displayed repetitive firing over the two compartments, with place fields in identical locations in each, but others did not. In a later experiment, Tanila (1999) recorded place cells while an animal moved directly in

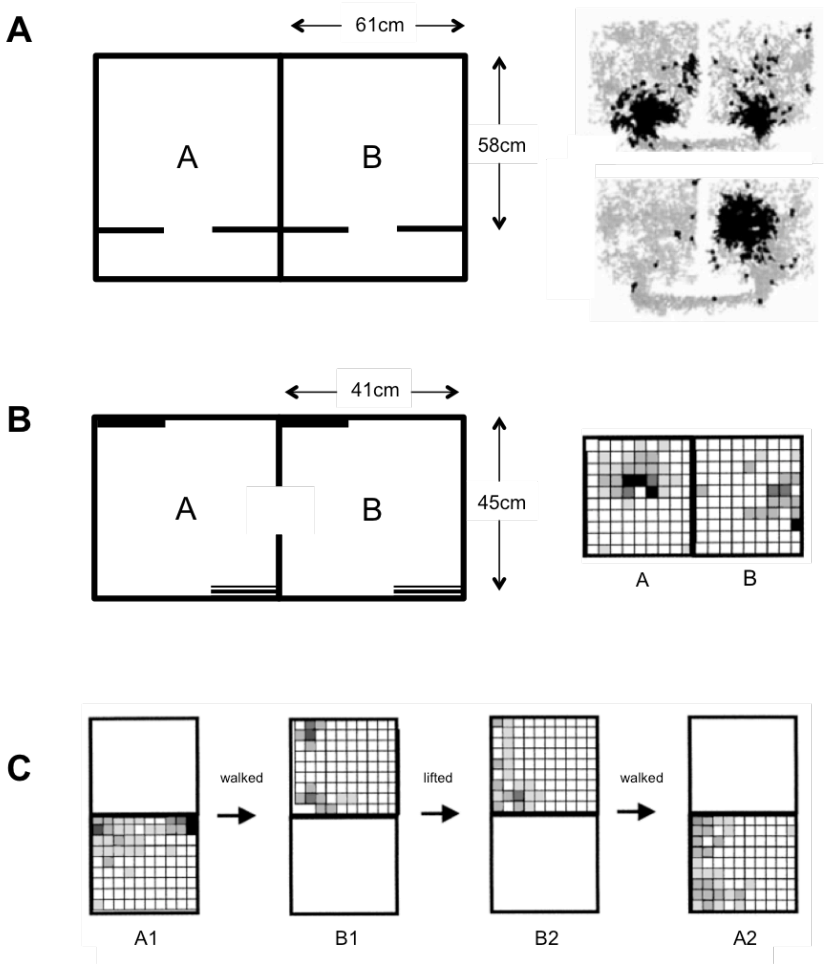


Figure 3.3: Place field activity in two identical compartments. (A) Left) Schematic diagram of the maze used by Skaggs & McNaughton (1998). Two visually identical compartments are oriented the same way and connected by a corridor. Right) Example place cells recorded in this environment, showing repeated (top) and remapped (bottom) fields. (B) Left) Schematic diagram of the maze used by Tanila (1999). Two visually identical compartments are oriented in the same way and connected by a doorway. Right) An example place cell recorded in the environment where the animal explored both boxes A and B, and represented them differently. (C) An example of a cell from Tanila (1999) that originally represented both boxes differently but when reintroduced to the environment by the experimenter to box B, maintained its representation upon returning box A. [Figures adapted from Skaggs & McNaughton 1998; Tanila, 1999]

between two identical compartments (A and B) via a central doorway (Figure 3.3B). He found that many cells formed new representations for boxes A and B upon moving between the two, but also that cells were also just as likely to reactivate their box B representation as reactivate the original box A representation on return to box A (Figure 3.3C). Both of these experiments noted the disconnect between some place cells representing each discrete compartment in the same way, while the animal's PI information reports that it is moving in between the two. This disconnect could be explained by the fact that grid cells, thought to represent the cognitive map's distance metric and be a readout of PI information (Fuhs and Touretzky, 2006; McNaughton et al., 2006; Moser et al., 2008), seem to lose their characteristic grid firing pattern in a repeating alleyway 'hairpin' maze (Figure 3.4) and instead fire in similar locations in each alley (Derdikman et al., 2009). Alongside loss of the grid pattern in this maze, Derdikman et al. (2009) found that place cells also had similarly repeated fields. This led to the conclusion that instead of forming a global grid-firing pattern, these cells were reduced to responding to purely local cues *i.e.* the distance moved since a turn into the

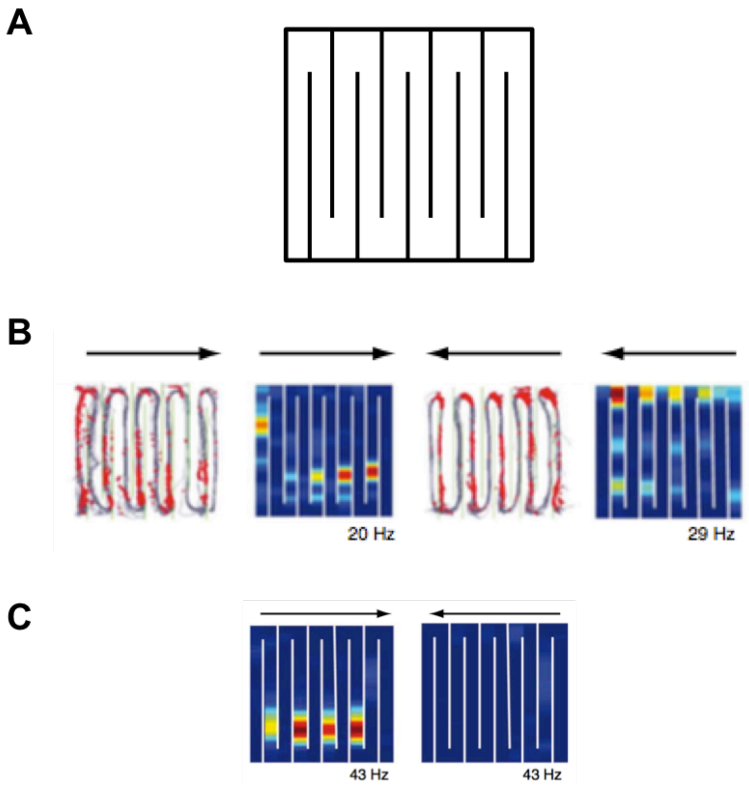


Figure 3.4: The apparatus used in Derdikman et al., 2009. (A) Schematic of the 'hairpin' maze: rats are trained to run from one end of the maze, through the sequence of alleyways to the other end and then back to the start continuously. (B) An example grid cell firing rate map; the grid cell fires at a similar location in multiple alleyways when the animal is running in a particular direction through the maze. Arrows above indicate the direction the animal is travelling. (C) An example place cell map showing a cell that again fires repetitively over multiple alleyways, though here only when the alleyways are facing in the same direction. Place cell firing is unidirectional in this maze, such that the cell only fires when the rat is travelling in one direction. [Figure adapted from Derdikman et al., 2009]

alleyway thus presenting as multiple firing fields in equal positions in each alley. Given that place cells then share this firing behaviour, it has been suggested that when the grid representation becomes fragmented like in the hairpin maze then the entorhinal/path integration input into the hippocampus may then influence the place cells to have repeated fields. If grid cells really are the neural basis of path integration, this type of fragmentation (causing the spaces to only be locally encoded with no reference to a global environment) may then explain why animals are unable to resolve similar compartments as separate despite consciously moving between them.

Building on this knowledge of grid cell fragmentation, Spiers et al. (2015) asked whether animals could differentiate multicompartment environments with numerous compartments more or less easily than the original two-box condition used by Skaggs & McNaughton (1998) (Figure 3.3A). It was thought that a greater number of compartments would provide more obvious PI information on moving between areas, but if grid cells re-tuned to represent local compartments only as in Derdikman et al., 2009 then place field repetition would still be present. To test this, place cells were recorded while rats freely-explored a four-compartment maze (Figure 3.5); here, it was found that instead of firing in a global manner (with one localised place field), the cells instead had repeating place fields in two or more compartments more often than by chance. This suggested that, similarly to Derdikman et al. (2009), the firing of these

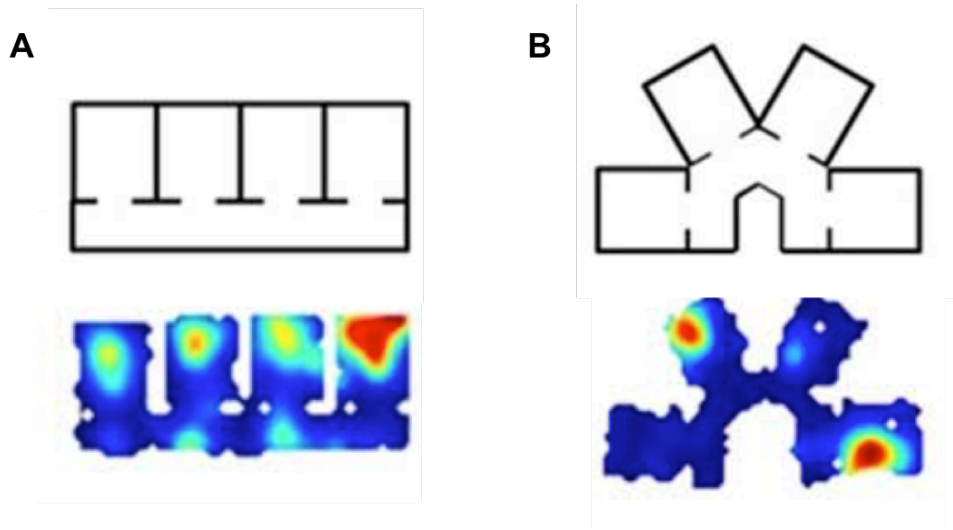


Figure 3.5: Place cell activity in multicompartmented space. (A) Top) Schematic of the four-compartment maze used by Spiers et al. (2015) and Grieves et al. (2016), consisting of four visually identical compartments connected by a corridor. Bottom) Place field activity in the four-compartment maze, showing repetitive firing in the maze. The incidence of repetitive field activity in this maze, in both studies, was higher than expected by chance. (B) Top) Schematic of the four-compartment radial maze used by Grieves et al. (2016), where the compartments were the same look/size as the parallel version but each oriented at an offset of 60° to one another. Bottom) Place field activity in the radial maze does not repeat, suggesting that the directional system can help distinguish cases of spatial ambiguity. [Figure adapted from Grieves et al., 2016].

place cells reflected local environmental cues in each compartment rather than the global environment. Further manipulations found that disrupting the spatial context (e.g. changing the size or colour of one of the compartments; Anderson and Jeffery, 2003; Paz-Villagran et al., 2004) could cause a break in repetition, but that these changes only occurred in the altered compartment. The authors suggested that repetition may be a result of grid fields resetting in the doorway to each compartment, and thus within each compartment place cell firing is dependent on local cues. Both grid fields (Marozzi et al., 2015) and place fields (Anderson and Jeffery, 2003) are known to alter their firing purely in response to changes in spatial context, explaining why how a single locally represented compartment could cease to have repeated firing without affecting the others if the spatial context was changed.

Place field repetition appears to occur when similar environments are oriented in the same direction as one another, but occurs to a much lesser extent (or not at all) in cases when similar environments are oriented in different directions. Evidence that different orientations can drive higher discrimination of similar environments comes studies by Fuhs et al. (2005) and Grieves et al. (2016). In the former, two visually identical compartments placed in opposite directions (*e.g.* North vs. South) were found to induce a high proportion of place cells to remap between sides, while a higher incidence of place field repetition was found when both compartments faced the same direction (*e.g.* both North). The latter study introduced a 60° radial offset between compartments to a four-compartment apparatus similar to that of Spiers et al. (2015) (Figure 3.5); they found that while place cells indeed showed repetitive firing in the parallel version of the maze, the same cells remapped between the compartments in the radial version (Grieves et al., 2016). Taken together, these results suggest that the directional system could be used to resolve spatial ambiguity.

3.4 HD system and directional ambiguity

Current studies of retrosplenial cortex (RSC) head direction cells in two connected but visually rotated compartments suggest that there is a mixed response to the conflict created by visual inputs and the global directional signal (Jacob et al., 2016; a study contributed to by the author, D. Overington). These cells were evaluated in a rotationally symmetrical two-compartment apparatus, distinguished by odour (the same apparatus used in this thesis, see section 4.4.1); some cells displayed a global encoding with consistent PFD across compartments, while others' PFD 'flipped' 180° as the rat moved from one compartment to another (Figure 3.6A). The authors proposed that the head direction cells presenting classical unipolar tuning curves were able to use the odour context cues to maintain consistent firing over the whole

apparatus and provide directional information (Figure 3.6A). In contrast to this, the cells presenting two well defined head direction PFDs separated by 180° ('bipolar' cells) were dominated by local visual cues, and thus informed only on the local reference frame and did not provide a global 'sense of direction' (Figure 3.6A). Interestingly, head direction cells recorded from the ADN and PoS under the same protocol only displayed the classical global firing pattern (Figure 3.6B, Appendix I).

The fact that these responses are mixed (*i.e.* some cells display bipolar activity and others do not), and that HDC recorded from other brain areas in the same apparatus consistently are consistently unipolar, suggests that the inconsistent RSC head direction cell population may not belong to the same proposed attractor network. This prompts the question of which signal, local or global, leads to the absolute 'sense of direction' that informs other spatial areas like the hippocampus; and also leads to the hypothesis that the conflict between these two populations could be a way that the spatial system resolves directionally differentiated visually ambiguous spaces.

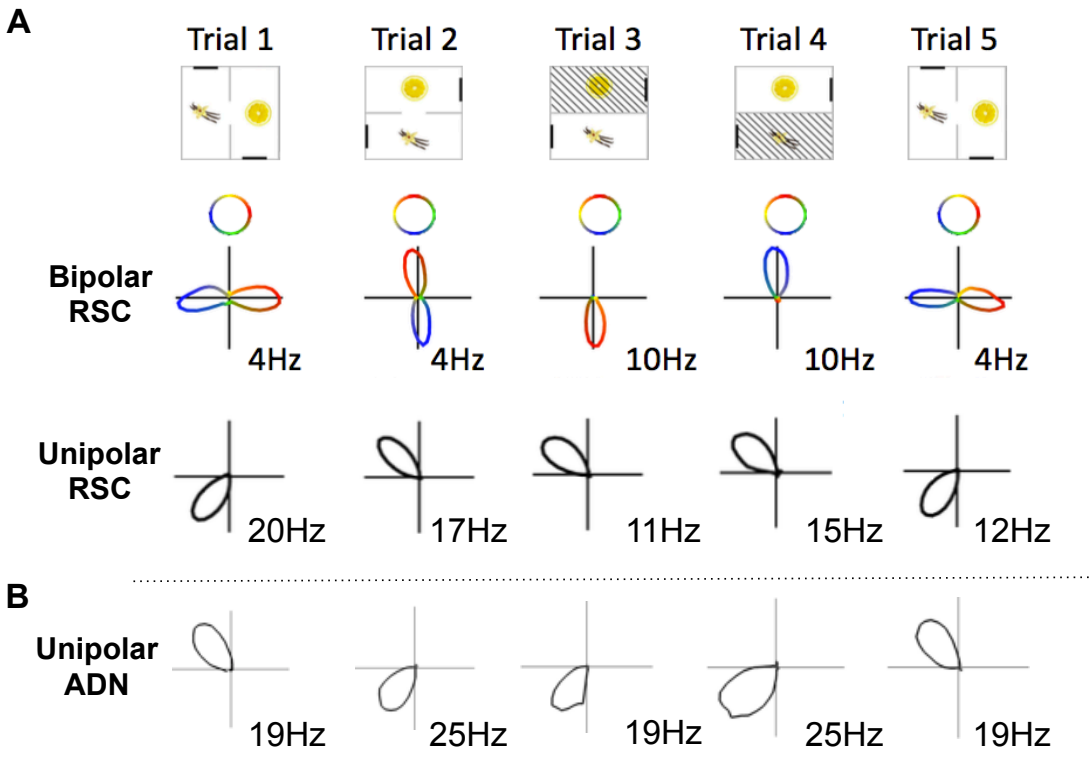


Figure 3.6: Two types of head direction cell responses to identical but visually rotated environments. (A) Top) Schematic of the two-compartment apparatus, with the orientation of the environment in each trial. (cross-hatching = unavailable compartments). Middle, Bottom) Polar plots of the cell's firing rate as a function of the head direction, with a circular colour key above the plot. Number = peak firing rate. Plots shown for bipolar and unipolar RSC head direction cells. [Figure adapted from Jacob et al., 2016]. (B) Polar plot for a unipolar ADN head direction cell in the same protocol recorded by the author (D.Overington), see also Appendix I; ADN head direction cells display 'classical' directional firing in the global reference frame.

3.5 Summary

It is clear that place cells, grid cells and head direction cells of the cognitive map are able to use both visual landmark information and information about the spatial context to modulate their representations of space. However, it seems that place cells are poor at resolving the ambiguity of similar spaces and often represent them in the same way, resulting in repetitive fields (Spiers et al., 2015). This place field repetition can then be broken with the addition of directional information as a distinguishing factor (Fuhs et al., 2005; Grieves et al., 2016).

The guiding hypothesis of this thesis is that the two types of directional firing present in the retrosplenial cortex (Jacob et al., 2016) may allow an animal to disambiguate visually identical compartments. To explore this, the experiments in this thesis aim to:

1) Ascertain whether behaving animals can resolve the directional ambiguity of the two-compartment context box using odour context information.
2) See how place cells respond to this visually ambiguous environment, and whether the directional information at the hippocampal level is consistent with global or local direction encoding.
3) Explore if (and how) the head direction system is involved in this resolution of directional ambiguity.

Chapter 4 - General Materials & Methods

The experimental works presented in this thesis belong to one set of behavioural experiments, with (Chapter 7) and without (Chapter 5) pharmacology, and one set of electrophysiology experiments (Chapter 6).

4.1 Animals

All procedures were licensed by UK Home Office subject to the restrictions and provisions contained in the amended Animals (Scientific Procedures) Act 1986 (2010/63). All animals used were adult male Lister Hooded rats of at least 8 weeks of age and 350 grams in weight before experimental procedures or surgery. Animals were housed in Perspex cages in humidity and temperature controlled conditions under a reversed 11:11 hour light:dark cycle with 1 hour (x2) of simulated dawn/dusk between 7-8AM and 7-8PM. Food and water were available *ad libitum* prior to any surgical procedures. After surgery, food was restricted to maintain 90% of free-feeding weight (minimum weight of 400g) with *ad libitum* access to water. Animals were not food restricted after surgery until a 7-day recovery period had elapsed.

In total, 27 animals were used in these experiments: 16 animals for behavioural testing; 3 animals for electrophysiological recordings with implanted microdrives; and 8 animals for inactivation studies with implanted cannulae.

4.2 Electrodes and microdrives

Rats were implanted with four or eight tetrodes (Recce and O'Keefe, 1989) held in a microdrive assembly (Axona Ltd, St. Albans, UK) – the difference in tetrode number produces either 16 or 32 channel microdrives. A precision screw in the microdrive assembly allows the tetrodes to be lowered or raised through the brain in steps as small as 25 microns (Figure 4.1). Tetrodes were made from four interwoven 25µm diameter platinum-iridium (H-ML insulated) wires (California Fine Wire, USA). Tetrodes were woven together with ~2 turns per millimeter of wire, with 7.5mm of each wire left unwoven, and the woven section sealed together by 45 seconds of heating with hot air. Approximately 5mm of insulation was removed from each of the four strands by heating with a paraffin flame. Tetrodes were then passed through a 12-15mm long 21-gauge cannula attached to the screw of the microdrive, and each strand connected to an individual microdrive channel. These wires were secured to the microdrive channels with conductive silver paint (Electrolube Ltd, UK), ensuring a strong electrical

connection between the two, and all four wire-channel sets were ultimately sealed with nail varnish for protection and insulation.

The protruding tips of the tetrodes were cut to the same length as each other with serrated surgical precision scissors (Fine Science Tools, Germany). This improved the amount of surface area available for each wire to conduct electrical activity from the brain, and allowed the activity collected by each electrode of a single tetrode to be compared against one another thus allowing isolation of single cells.

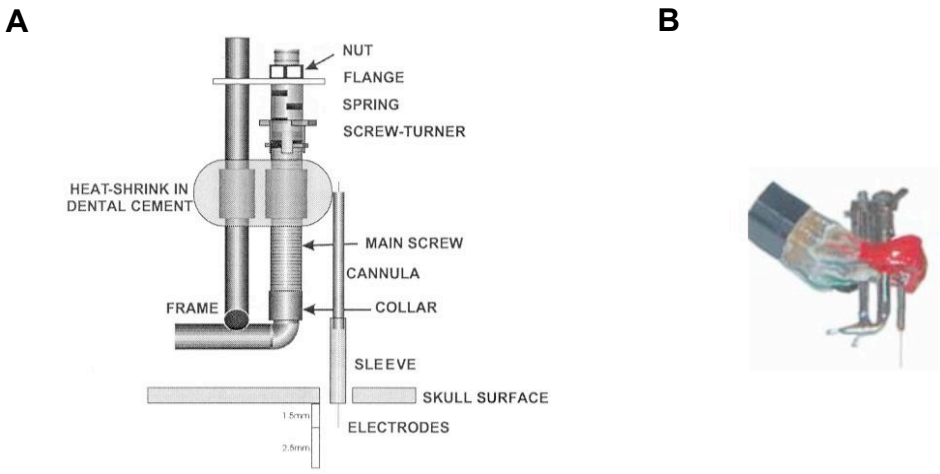


Figure 4.1: Schematic diagram of the microdrives used in this thesis. (A) Schematic of the drive design; tetrodes are threaded through the cannula and implanted into the brain. They are protected by the sleeve once implanted. (B) Microdrive ready to implant; tetrodes are protected with nail varnish (red), and can be seen protruding beneath the frame. [Images courtesy of Axona Ltd., St Albans, UK]

4.3 Surgical procedures

Some animals received one of the following procedures: implantation of movable tetrodes into the brain for electrophysiological neuron recording or implantation of fixed cannulae for infusion of drugs. Coordinates for implant or injection sites are shown in Table 1:

	Anteroposterior (mm)	Mediolateral (mm)	Dorsoventral (mm)
HPC [Implant]:	- 4.00	+/- 2.50	- 1.50
ADN [Implant]:	- 1.80	+/- 1.30	- 3.70
ADN [Cannula]:	- 1.90	+/- 1.30	- 3.20

Table 1: Injection and implant coordinates relative to bregma (in mm). Injection and implant sites for all the procedures carried out in this thesis. Coordinates varied between procedures even targeting the same structure.

4.3.1 Preparation

All surgical tools were sterilized in an autoclave or disposable sterile items were used where appropriate, and aseptic technique was used throughout. After weighing the animal, anaesthesia was induced via an induction box using 3% isoflurane (O_2 3L/min). After loss of pedal withdrawal reflex, the hair over the skull was clipped and lubricant applied to the eyes (Viscotears, Novartis Pharmaceuticals Ltd). The animal was placed in a stereotaxic frame (Kopf, USA) and anaesthesia was maintained from this point via face-mask with 1.5-3% isoflurane (O_2 3L/min). Prophylatic analgesic Carprieve (0.1ml of 1:10 dilution in saline per 100g animal weight, Norbrook) was administered subcutaneously prior to surgery start. The skin at the surgical site (the animal's head) was cleaned with iodine solution before an incision was made along the midline over the skull and the retracted skin held in place with haemostat clamps. The periosteum was removed in a craniotomy, and the skull area cleaned and dried. The stereotaxic frame and arm enabled the precise localization of the microdrive/cannula/needle position relative to bregma and lambda, and allowed confirmation that the skull was flat in the dorso-ventral axis (Paxinos and Watson, 1982).

4.3.2 Microdrive implantation

In addition to the craniotomy performed at the region of interest (Table 1) in *Preparation*, six further holes were drilled into the skull with a burr drill and six jewellers' screws were screwed into them. One screw was soldered to a ground wire and lowered until touching the surface of the brain. This ground wire was later connected the microdrive to ensure that the rat was electrically grounded. A durotomy was performed at the site of the craniotomy, and the tetrodes were inserted at the appropriate coordinates relative to bregma. Care was taken to ensure the tetrodes did not bend as they were inserted into the brain. The exposed tetrodes were protected by a metal guide cannula, and sterile Vaseline (Unilever, USA) placed around the cannula to cover any exposed brain tissue. Dental acrylic was then carefully applied around the base of the microdrive 'feet' and guide cannula in order to firmly attach the drive to the skull – the screws act as anchoring points for the dental acrylic (Simplex Rapid ®, Kemdent, UK). Once the cement was set, the ground wire was soldered to the microdrive.

4.3.3 Anterior thalamus cannulation

For acute administration of drugs to the anterior thalamus, rats were implanted with 26-gauge cannulas (Plastics One, Bilaney, UK) into the area above the anterodorsal thalamus (Table 1). Procedures for cannula implantation were the same as in 4.3.2 except that only four jewellers screws were used instead of six. Cannulas were cut to

9mm and sterotaxically implanted so that the tip was located 1mm above the target site. A 33-gauge stylet cut flush with the 26-gauge implant was placed into the cannula to prevent collection of debris. Cannula placement was confirmed in fixed and sliced tissue after the experiments.

4.3.4 Post-operative care

Post-surgery, animals were monitored periodically until they woke. Meloxicam (Metacam, 0.2mg/kg, 0.3g/day, Boehringer Ingelheim UK) in condensed milk was given as a post-operative analgesic for 2 days. At least 7 days were allowed for recovery before further experiments, and during this time food and water were available *ad libitum*. Animals were individually housed after surgery and throughout experiments, with increased cage enrichment.

4.4 Experimental procedures

Experimental procedures specific to particular studies will be detailed in their respective chapters; what follows are technical methods and shared procedures.

4.4.1 Experimental apparatus

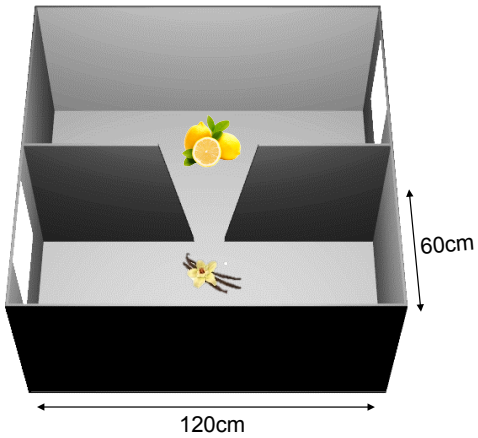


Figure 4.2: Two-compartment context box apparatus. This apparatus consisted of two visually identical but oppositely oriented boxes, dimensions of 120 x 60 cm each. Thus, from the point of view of the animal, the boxes were visually identical but reversed in orientation when the rat stood at the central door. One compartment was scented with lemon odour while the other was scented with vanilla.

The two-compartment experimental apparatus was used for Experiments 1, 2, and 3; two separate boxes were constructed to the same specifications for parallel data collection. The square recording environment was constructed from black medium fiber density (MDF) board (120 x 120 x 50cm), with the entire interior (floor and walls) lined with black vinyl flooring to provide a wipe-clean surface (see Figure 4.2). A dividing wall separated the arena into two visually identical rectangular compartments (120 x 60cm), with an 18cm opening in the dividing wall acting as a doorway between the two. Four

overhead LED lights provided diffuse light sources. Each compartment of the box was dedicated to the lemon or vanilla odour throughout the experiment (i.e. across all rats) – before each trial, the vinyl floor and walls of the box were scented with lemon or vanilla odour by applying 2ml of lemon or vanilla food flavouring (Supercook, UK) onto a pre-moistened sponge and wiping the surfaces as uniformly as possible. Between trials any sawdust, faeces or urine from the animal were removed with a small amount of ethanol before refreshing with the corresponding odour.

4.4.2 Behavioural experiment procedures

4.4.2.1 Video tracking

Locomotor activity of the animal was recorded from an overhead Logitech webcam (Switzerland) using DacqTrack software (Axona Ltd, St Albans, UK). Non-toxic orange colour (Uni Posca, UK) was applied to the skin of either the head (shaved before the experiment) or the back of the animal to facilitate positional tracking. Positions of the animal were recorded using the RGB tracking facility and converted into x,y coordinates; this was achieved by recognition of pixels in the red range of the webcam feed (localised to the animal's head or back) and finding the averaged centre of these pixels to give an x,y coordinate of the animal's position.

4.4.2.2 Data analysis

Object exploration over trials and days

The raw object exploration data were analysed using one-way, two-way and repeated measures ANOVA, and the 2-tailed t-test.

To assess differences in exploration over trials and days, one-way and repeated measures ANOVA were performed. To further analyse any potential learning trends in the behavioural data, a Jonckheere trend test was used (Jonckheere and Bower, 1967). The Jonckheere test is a rank-based non-parametric test that can be used to determine if there is a statistically significant trend in medians between an independent variable (in this case, trials / days) and a dependent variable (object exploration), and requires the independent samples to be orderly arranged (in this case trial 1, trial 2, etc). This test was applied here to look at learning within a given experimental day (the whole session for a pilot, or one day in the full paradigm) or across the full experiment (five experimental sessions, one per day); it tests whether there is no *a priori* ranking of the medians of the independent variable (trials / days), thus whether there is learning over time. The test was only performed on habituation trials, as the manipulation in the probe trial was likely to influence animal behaviour.

To assess learning within a single day, the standard J-T statistic (individual *z* score) was generated per animal for a given experimental day by comparing trial pairs (equations for the J statistic and standard J-T statistic are as below; for greater detail see Jonckheere & Bower, 1967). To give a group averaged *Z* score to show the trend in a single day, the individual *z* scores were summed and divided by the number of animals (N, as per the third equation below). To assess learning over the full experiment, *z* scores were produced from the average exploration of each animal on each day (*i.e.* one *z* score per day per animal), then summed and divided by the number of animals (N, as per the third equation below) to give a group *Z* score for the full experiment. The 2-tailed *Z* scores were then compared to a normal distribution to obtain a significance value.

Equations:

$$JT\ statistic = \sum_{k1=1}^{K-1} \sum_{k2=k1+1}^{K} U_{k1k2}$$

where k is the number of samples (k=1, 2, …, K) and U is the Mann-Whitney U count for each sample pair.

$$standard\ JT\ statistic = {JT\ statistic - expected\ JT\ statistic}\Big/{variance\ (JT\ statistic)}$$

where the expected J-T statistic is the J-T statistic if the null hypothesis is observed.

$$Z = {\sum_{i=1}^{N} z_i}\Big/{\sqrt{N}}$$

where *Z* is the group trend score, and *z* is the standard J-T statistic per N animals.

Exploration of displaced and non-displaced objects

Differential exploration of the displaced and non-displaced objects in the probe trial was first assessed using the D2 analysis of discrimination (as per the equation below, Ennaceur and Delacour, 1988). This analysis reflects the discrimination of the animal between novel and familiar objects.

$$D2 = {(b-a)}\Big/{(a+b)}$$

In this analysis, (a) referred to the time spent exploring the non-displaced object in the probe trial, and (b) referred to the time spent exploring the displaced object. For the habituation trials, (b) was assigned to the object in the odour that would later be

displaced in the probe trial *i.e.* the probe trial displaced the object in the vanilla odour, so the object in the vanilla odour was assigned (b) for habituation trial analysis.

To further assess discrimination of the objects, a novel 're-exploration score' was used. This measure, based on a similar analysis by Van Cauter *et al.*, (2013) aims to take into account the behaviour of the animal across the habituation and probe trials. In this measure, the time spent exploring each object was separately calculated as a proportion of the average baseline exploration of that object (baseline exploration = average exploration time of the object in the 7 prior habituation trials).

$$Reexploration\ score = {}^{object\ exploration}/_{average\ exploration\ of\ object\ in\ T1-7}$$

4.4.3 Electrophysiology experiment procedures

4.4.3.1 Single unit recording

Animals were placed onto a holding platform, where the microdrive on the animal's head could then be connected to multichannel recording equipment (Axona Ltd., St Albans, UK) via a headstage with 2 LEDs and 4m of lightweight wires. The LEDs (located on the headstage of the animal) captured the position of the rat that was tracked by a camera mounted on the ceiling, directly above the apparatus; these LEDs were of different intensities so the angle between the 'big' and 'small' spot could be used to capture the direction of the animal's head. Position of the LEDs was captured by the camera and converted into x,y coordinates.

The local field potentials (LFPs) recorded from each of the channels were passed through an RC-coupled, unity gain operational amplifier, which was mounted on the animal's head. Each channel was amplified approximately 10,000 to 20,000 times and band pass filtered (500Hz to 7kHz). Each tetrode was differentially recorded against a channel on a different tetrode ('referenced'), and one channel was used to record the LFP. Each channel was sampled at 50kHz but only action potentials which fired at a rate higher than a user-defined threshold were collected and saved to disk for offline analysis. Action potentials firing above the threshold were collected for a total of 1ms (0.2ms pre-threshold and 0.8ms after). Activity on each channel was visualized using a single unit oscilloscope display and could also be listened to via an audio amplifier.

4.4.3.2 Screening protocol

Animals were screened in a quiet laboratory room, inside a 1mx1m square environment with 0.75m high plywood walls. No curtaining was present around the environment so many orienting cues were available to the animal *i.e.* black cue cards

on the environment wall and experiment room wall, various computer and experimental equipment, as well as various sounds and smells. Animals were encouraged to move around and sample the environment by foraging for scattered rice. Tetrodes were lowered in steps of 50 -100 microns per day until spatially modulated cells were found. Animals were taken back to their home cage for at least four hours between screening sessions.

4.4.3.3 Cell isolation

Cluster cutting software (Tint, Axona Ltd) was used to isolate single units from the multichannel recordings. All four channels of a tetrode were loaded simultaneously, and the software then plotted the peak amplitudes of spikes from each channel against the peak amplitudes of other channels from a given tetrode. This gave six separate cross-channel scatter plots of spike amplitude (grey dots, termed 'cluster space'; Figure 4.3A). Comparing the waveform across all 4 electrodes is comparable to performing triangulation of that signal in space (the extracellular space is assumed to be homogenous for this process). The amplitude and shape of spikes differ between electrodes due to a difference in the distance between a cell and a single electrode; cells more proximal to the electrode appear to exhibit higher spiking amplitude. It is thus possible to cluster spiking amplitudes, allowing for isolation of distinct single cells. Confirmation of whether a given cluster was a result of neural activity was done through inspection of the average waveform of a cluster (Figure 4.3B). A cluster was considered to be a single cell's activity if the waveform had characteristics of an action potential (i.e. an initial peak followed by a refractory period).

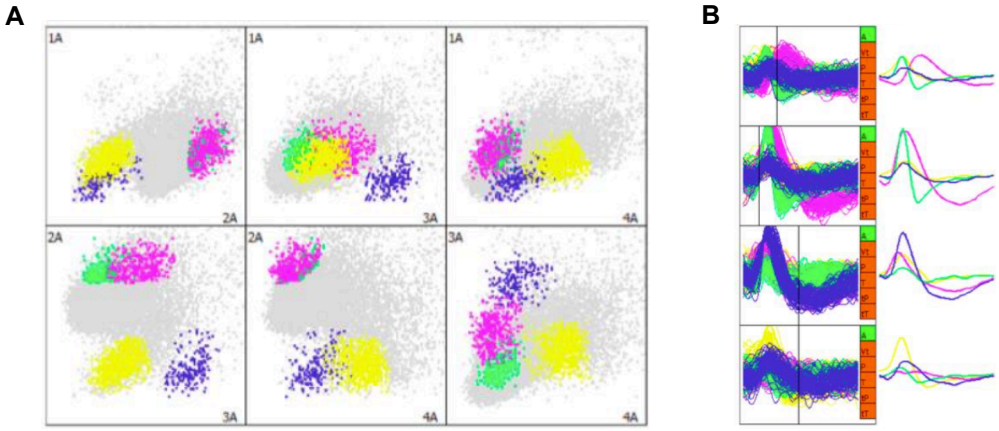


Figure 4.3: Example screenshots from cluster-cutting software Tint (Axona, St Albans, UK). (A) Electrode channel-pair scatter plots comparing the peak-to-peak amplitude of each spike on one channel against the amplitude of that spike on other channels. Superimposed on this in colours are the clusters of spikes selected manually by the experimenter. (B) Plots showing 100 superimposed waveforms from each cluster coloured in A (left), and the mean waveform for each cluster on each channel (right). The colours consistently refer to the same cluster.

4.4.3.4 Place cell identification

Once single cells had been isolated, the spatial activity was assessed for all clustered cells visually. This was done by assessing occupancy normalized rate maps and spiking maps for regular / spatially restricted firing. Rate maps were created for each isolated cell by binning the data into 2.5cm x 2.5cm bins and smoothing with a 5x5 bin boxcar algorithm. Smoothing replaced the value of each bin with the average value of the bin plus the surrounding eight bins. Firing rate for each bin was calculated by dividing the number of spikes that occurred in each bin by the amount of time the rat spent in it. The peak rate for a cell was taken from the bin with the highest firing rate (in Hz) for a given trial. Each rate map was displaced as a false colour density map with each colour ranging from blue to red representing a 20% band (e.g. red 80-100%; blue 0-20%, as in Figure 2.3). Populations of putative place cells were identified by assessing spatial activity of each cell's rate map by eye. Cells in this population that fired over more than 50% of the total environment area or did not reach a peak-firing rate of 1Hz in baseline trials (Experiment 2, trials 1, 2 and 5) were then excluded from further analysis.

4.4.3.5 Spatial correlations

The similarity of spatial firing between trials was assessed by computing a spatial correlation between the two trials' smoothed rate maps (as in Spiers et al., 2015). For this, the Pearson product moment correlation coefficient was calculated between spatially equivalent bins (2.5cm x 2.5cm) in the two trials. Unvisited bins as were excluded from this calculation to prevent artificially high correlations. Cells were deemed to be stable the two trials if the spatial correlation was above a determined remapping threshold (0.30; for how the threshold was obtained see 4.4.3.6) and unstable if the spatial correlation was lower than the threshold. Cells that were deemed unstable between the trial 1 and 5 baselines were analysed as a separate population.

4.4.3.6 Determination of inter-trial remapping threshold

In order to determine whether a cell's spatial representation remained the same between two trials within the same recording session, the spatial correlation between the rate maps of those trials was computed (as described in section 4.4.3.5). The spatial correlation indicated how similar the representations were, and it was necessary to determine a threshold above which one could consider that the representations remained stable. Some publications use a user-defined arbitrary threshold for remapping, usually inferred by eye (e.g. a threshold of 0.4 is used by Anderson & Jeffery, 2003 and Spiers et al., 2015). However, the experiments here involved a non-conventional recording environment as well as trials in which would test the spatial

system's ability to recall parts of an environment post introduction of barriers. Hence it was important objectively obtain thresholds for remapping based on real data.

In order to obtain an estimate of how well cells' firing patterns correlated by chance, a shuffled distribution was created by correlating cells' rate maps in the trial 1 baseline with different cells' rate maps in the trial 5 baseline (see Figure 6.1 for experimental protocol). This distribution included all cells recorded from all rats. The null distribution and resulting threshold (95th percentile of the shuffled distribution) is shown in Figure 4.4. For a place cell to be considered spatially stable in a given recording session, the rate maps needed to show a spatial correlation above 0.30. This value corresponded well with the experimenter's subjective judgement of stability looking at the rate maps by eye.

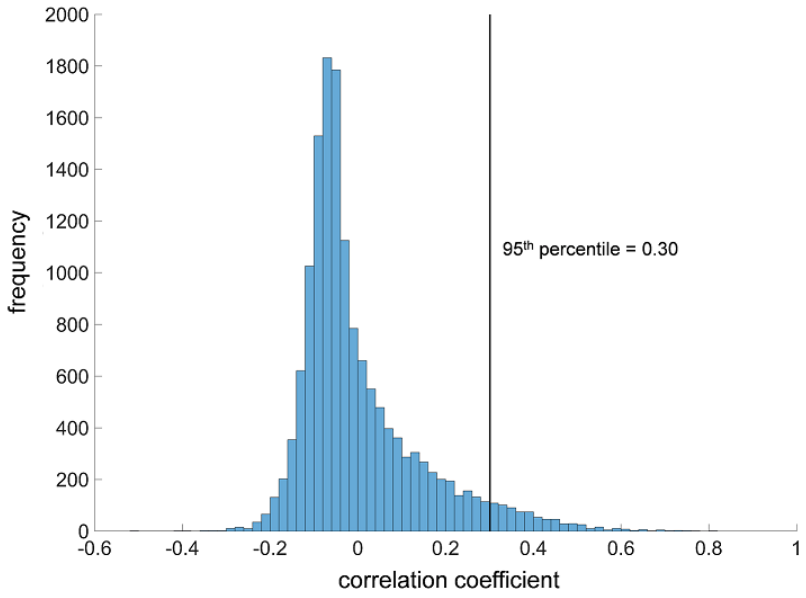


Figure 4.4: Shuffled distribution used to obtain spatial correlation remapping thresholds between trials of a given recording session. Histogram of the shuffled spatial correlations between trial 1 rate maps and trial 5 rate maps, with a 95th percentile (and thus remapping threshold) of 0.30; the correlations between the rate maps of trial 1 and 5 from the same cell have been removed. 11327 correlations were included from 122 unique place cells.

4.4.3.7 Between-trial and between-compartment comparisons

Spike cluster separation was performed on each of the recording session data for a given experimental day (trials 1-5), and rate maps were produced for each cell in each recording trial as described in section 4.4.3.4.

Once the rate maps were obtained and assessed to pass the 50% coverage criteria and previously calculated stability threshold between trials 1 and 5 (0.30, see 4.4.3.6), further spatial correlations were then performed: baseline trials compared to their single compartment counterparts in closed door trials; and half-map comparisons (where the

rate map was divided in two post-hoc, keeping whole compartments) between compartments in baseline trials.

Between-trial correlations were carried out as follows (Figure 4.5): the rate map for trial 1 was generated and stored, then the rate map for the trial to be correlated (trial 2, 3 or 4) was stored separately. The angle of rotation between the two rate maps was then calculated based on the rotation of the experimental apparatus, and the second rate map was rotated so that it matched the orientation of first. In the case of correlating trial 1 with the 'closed door' trials (3 or 4), the dimensions of the map to be correlated were also extended to match by padding the matrix in the respective dimension with NANs.

To assess whether there were patterns to place fields within the two-compartment box, between-compartment correlations were carried out as follows: the baseline rate map for trial 1 was split in half and the two halves were spatially correlated against one another at 0 and 180 degrees. The rate map was always halved such that the split separated the two-compartment space into the component sub-compartments, regardless of the orientation of the apparatus.

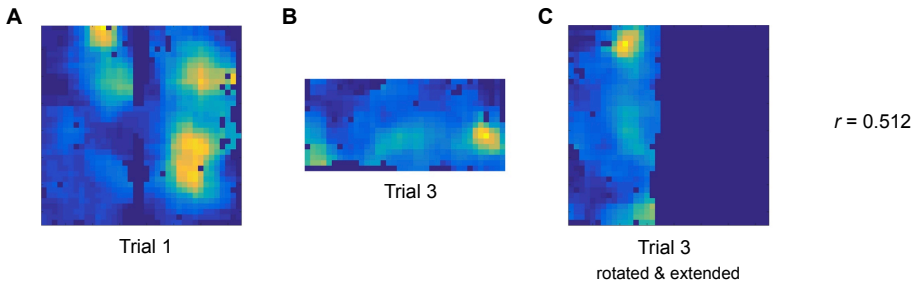


Figure 4.5: Process of between trial correlations for closed door trials. (A) The rate map of place cell activity in trial 1 is generated and stored. (B) The rate map for cell activity in the closed-door trial 3 is generated and stored. (C) The rate map in trial 3 is then rotated to match the orientation of the corresponding compartment in trial 1, and the rate map matrix is extended with NAN to match trial 1 compartment placement. The spatial correlation is then performed with NANs excluded from the correlation such that only activity in the desired compartment will be compared; for this cell, the firing activity of trial 3 vs trial 1 had a correlation coefficient (r) of 0.512.

4.4.3.8 Place Fields

For clusters identified as place cells, firing rate maps were used to separate distinguishable place fields that were present in the environment. Custom MATLAB scripts were used to isolate areas of firing over 40% of the maximum firing rate (peak firing rate). Regions of firing meeting the criteria that also had an area greater than 9 contiguous pixels (1 pixel = ~2.5cm^2) were considered place fields. The size, position and firing rate properties of these fields were then extracted.

4.5 Ex vivo brain analysis

4.5.1 Perfusion fixing

At the end of the experiment, rats were deeply anaesthetized with pentobarbital (Euthatal 1ml/100g, Merial, UK) and following loss of the pedal reflex, the chest cavity was opened to reveal the heart. A needle was inserted into the tip of the left ventricle and the right atrium was cut open. In some animals the descending aorta was also clamped so only the upper half of the body would be fixed. Saline was infused at 2ml/min and, when the exsanguinate ran clear, was exchanged for 10% formalin (Sigma Aldrich, UK). The whole brain was then carefully removed and post-fixed overnight at 4°C in 10% formalin, before cryo-protection in 30% sucrose in 1% phosphate buffered saline at 4°c for a minimum of 48h.

4.5.2 Histology

Fixed and cryo-protected brains were mounted on a pre-chilled platform with Tissue-Tek O.T.C. (Sakura, UK) and left to freeze. 30µm slices were sectioned coronally using a cryostat (Leica CM1850 UV) at -20°C, directly mounted onto SuperFrost slides (Thermo Scientific, USA) and left to equilibrate overnight at room temperature. Brains from cannulated animals receiving infusions of fluorescent BODIPY-muscimol (Thermo Scientific, USA) (see section 7.1.6 were coverslipped with VECTASHIELD anti-fade medium (with DAPI, Vector Laboratories, USA). Brains from microdrive-implanted animals were stained with the cresyl violet method (stepping slides through an ascending alcohol series, a descending alcohol series, immersion in cresyl violet solution, adjustment with glacial acetic acid, and a final ascending alcohol series) to verify electrode placement. Slides were then coverslipped with DPX mounting medium (Sigma Aldrich, UK).

4.5.3 Imaging

Images of electrode tracks from cresyl violet stained slices were taken with an Olympus microscope with XLi digital camera (XL Imaging Ltd, Germany). These images were used to determine the location from which cells were recorded.

Slides with fluorescence were imaged on a Leica DMi8 fluorescent microscope under the red and blue channels (visualizing BODIPY-muscimol fluorescence and DAPI respectively).

Chapter 5 - Experiment 1

Can rats use context cues to resolve spatial ambiguity?

As described in Chapter 3, previous studies have demonstrated that contextual information can be reliably used by behaving animals to solve tasks requiring configural processing like the object-place-context task (Davis et al., 2013; Eacott and Norman, 2004; Langston and Wood, 2010). In such tasks, individual features of the environment are ambiguous, but are informative when integrated together as a configuration. Eacott & Norman (2004)'s object-place-context task was introduced as a method to test rats ability to demonstrate a memory for objects, their spatial position and the context in which they appeared.

The present experiment aims to use a variant of the object-place-context task (Eacott and Norman, 2004) as a tool to examine whether odour provides enough salient context information for the animal to resolve the directional ambiguity presented by the two-compartment apparatus (see Figure 5.1 and Jacob et al., 2016) and organise a spatial representation of location-odour based on landmarks. The guiding hypothesis is that a mismatch between local and global directional cues, as reflected by the two types of directional firing in the RSC, could allow an animal to disambiguate visually identical compartments. The design of the present experiment does not allow complete resolution of this question, but serves as a starting point, by asking: can olfactory context cues be used by rats to disambiguate visually identical spatial locations, when these are differently oriented?

To answer this, we developed a novel 'location-odour' task, based on the spontaneous object recognition paradigm (Ennaceur and Delacour, 1988), in the two-compartment context box. This apparatus (Figure 5.1) consists of two visually identical compartments with contrasting odours, each containing a polarising landmark (cue card), and connected by a door. The landmarks in this apparatus are directionally ambiguous without the context of the odour, and thus can only be informative if integrated by the animal as part of a configuration. During the habituation phase of the novel task, rats are

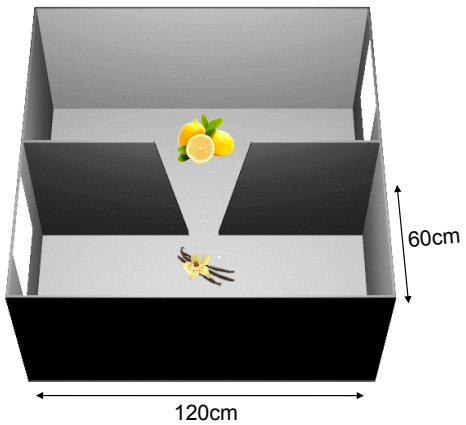


Figure 5.1: The two-compartment context box. This apparatus is visually symmetrical around the axis of the door; odour cues are present to disambiguate one compartment from the other.

presented with two objects in this apparatus; the objects were placed at contrasting distances to the landmark in each compartment (near to the landmark in one compartment, and far in the other); thus the positions of the objects can be used in an location-odour configuration to break the symmetry of the environment. However, in the single probe trial one object is spatially displaced (similar to Davis et al., 2013 and Van Cauter et al., 2013) such that location-odour configurations of both objects are the same and the environment once again has visual rotational symmetry. Subsequently, odour remains the only cue the animal can use to resolve the directional ambiguity of the landmarks; if the rat has encoded the olfactory information of each compartment together with the relative locations of the objects during the habituation phase, then the displacement of an object would create a novel 'location-odour' configuration. We then predict that animals would spontaneously increase their exploration of the object that appears in a novel configuration of odour and location relative to the landmark, over the object that was not displaced.

A preliminary version of this task, using animals that were very familiar with the two-compartment context box, indicated that the above hypothesis was correct. The series of experiments detailed in this chapter aimed to replicate these preliminary results in naïve animals: three pilot experiments (Experiments 1A, 1B and 1C) were carried out before the paradigm was refined for the final behavioural experiment (Experiment 1D).

As this chapter will show, the animals indeed explored the displaced object significantly more than the non-displaced, which indicates that they able to use odour to resolve the directional ambiguity of the landmarks and create distinct location-odour configurations in each context. Further chapters of this thesis will explore the hypothesis that this interaction between context and spatial cues is mediated by the head direction system.

5.1 Methods

5.1.1 Subjects

Six naïve adult male Lister Hooded rats were used in Experiment 1A, 1B, and 1C, and a further ten naïve adult male Lister Hooded rats were used in Experiment 1D. The animals were housed in groups of two or three in Perspex cages, with food and water were available *ad libitum*.

5.1.2 Experimental apparatus

The two-compartment testing apparatus (see section 4.4.1) used for this study was the same as in Experiments 2 and 3. Objects used in the experiments were identical square-based bottles (9 x 9 x 23cm); each was filled with sand and painted matte black. For testing in the third pilot and the full paradigm, the objects were enriched in texture and novelty (with Velcro strips on two opposite faces, and plastic attachments on the other two) but remained visually identical.

Circular opaque curtains surrounded the apparatus, creating an enclosure of diameter 170cm, so that no visual distal cues (*i.e.* external to the recording environment) were available to aid animal navigation. Local cues in the apparatus included: geometric relation of the doorway to each compartment; an A3 white cue card on the wall to the left of the door in each compartment (see Figure 4.2, or schematic in Figure 5.2) and the scent of the floor and walls. Encoding the location of the objects with relation to the landmarks and scent would provide a way for the rats to polarize the otherwise visually identical environments.

5.1.3 Behavioural testing

5.1.3.1 Pilot experiments - Familiarisation

Rats were individually familiarised to the arena for two days immediately prior to the start of each pilot experiment (Figure 5.2A). This part of the protocol is not to be confused with the single 'Familiarisation' trial on the experimental testing day. On the first day the animal was exposed to a 'clean' version of the apparatus without odours or objects in two 10-minute sessions, separated by a 60-minute inter-session interval. The second day consisted of the same 10-minute sessions but the specific odour contexts were introduced to each compartment of the apparatus (lemon and vanilla).

For each set of pilot experiments (Experiments 1A, 1B and 1C), both familiarisation and testing parts of the protocol were performed. Thus, the animals had varying levels of familiarity with the environment at the start of each pilot (see Figure 5.4, Figure 5.6 and Figure 5.8).

5.1.3.2 Pilot experiments – Testing paradigm

Each animal experienced ten trials on the experimental testing day, Figure 5.2B: re-familiarisation (trial 1), habituation (trials 2-9) and a final probe trial (trial 10). The previous familiarisation sessions and the habituation trials on testing day aimed to ensure that the animal was familiar with the 2-compartment apparatus, the odour contexts, and then the objects and their relative positions to the visual landmarks. Objects were always equidistant (25cm) from the three surrounding walls as well as from each other. Per animal, the distance of the objects from the landmark within each scented compartment (near/far) was counterbalanced such that for half the rats the objects were respectively close to the lemon cue card and far from the vanilla cue, and for the other half of the rats the inverse was true. Relative object positions were retained over the following sessions. The apparatus was cleaned with a 70% ethanol solution (Hydrex HS, Ecolab, UK) and re-scented with lemon and vanilla between animals.

For each trial the animal was placed in the centre of a randomly chosen compartment, and was allowed to freely explore the environment. Each trial lasted 4 minutes, with a 4-minute inter-trial rest in a holding box outside the curtained arena. During this rest period the arena was randomly rotated (by either 90 or 180 degrees, or not at all) in a

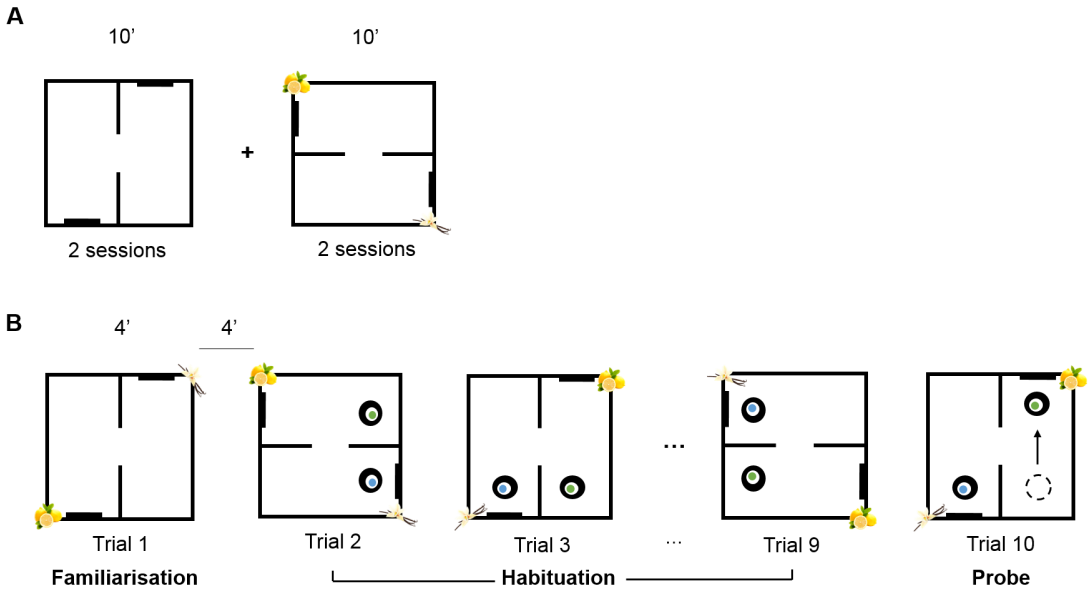


Figure 5.2: Behavioural testing protocol for a single animal (Pilots). (A) For the two days immediately prior to testing, animals were exposed to two sets of familiarisation sessions at a rate of one per day. The first day comprised two 10-minute sessions, separated by at least 60 minutes, of 'clean' box exploration *i.e.* with no odours. The environment was randomly rotated by factors of 90 degrees to eliminate possible use of extramaze cues. The second day comprised another two 10-minute sessions, separated by at least 60 minutes, of exploration of the apparatus with the specific odour contexts introduced. (B) The behavioural testing paradigm: Trial 1 was re-familiarisation to the arena with the odours present; Trials 2-9 were habituation trials with the odours and objects in specific relative positions; Trial 10 was the probe trial where the location of a single object was changed to render the environment visually symmetrical.

clockwise or anticlockwise direction, to remove influence of any cues not provided by the arena itself. Pairs of objects were randomly chosen from a set of four between trials and similarly handled to prevent odour differences etc.; this means that object recognition should be based purely on visual information.

Animal behaviour was captured by an overhead webcam and streamed live to a computer located in the experiment room but outside the curtained area. From this real-time video feed, animals were scored by the experimenter on their active exploration of each object in each trial. Scoring was manually recorded by a time-encoded keylogger (DacqTrack, Axona Ltd, St Albans, UK) from the live video stream of environment exploration. An acetate screen overlay indicated a 2cm radius around each object whilst the live-stream was active, and active exploration was defined as the rat's nose being within 2cm of and oriented towards the object, sniffing at, or otherwise closely attending to the object. This definition excludes using the object merely as support during rearing or sitting on the object.

5.1.3.3 Full paradigm – Testing session

Behavioural testing took place over five days, with four days of habituation only and a fifth day of habituation followed by test (Figure 5.3). Animals received 5 consecutive habituation sessions (eight trials each on Days 1-4 and seven trials on Day 5), at a rate of one session per day. These sessions were used to habituate the animals to the two-compartment apparatus, the contexts used in the study, and to the objects and their relative positions to the visual landmarks.

Object positions within the apparatus, object handling, testing procedures, arena rotations, and scoring were otherwise identical to as described in section 5.1.3.2.

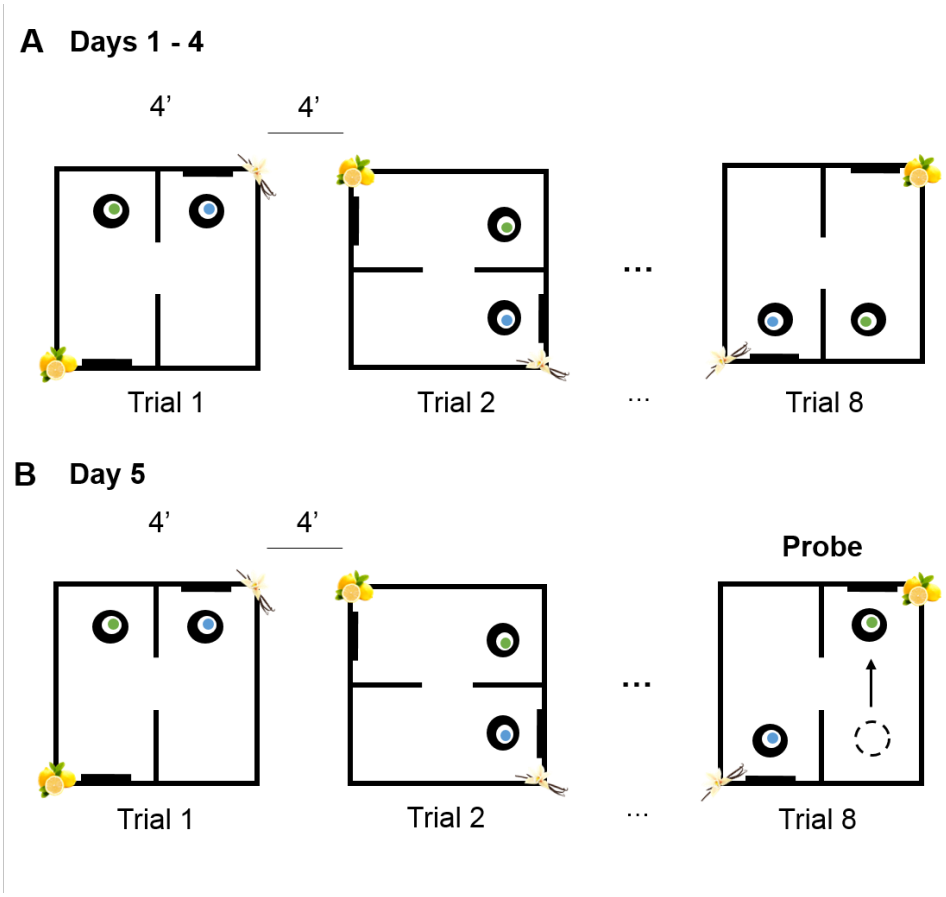


Figure 5.3: Behavioural testing protocol for a single animal (Full paradigm). (A) For the initial four days, animals were exposed to eight habituation trials of four minutes each per day, with an inter-trial interval of four minutes. The environment was randomly rotated by factors of 90 degrees to eliminate possible use of extramaze cues. This habituation included exploration of the apparatus with the specific odour contexts introduced. Objects were also placed in the arena, at the same global position in the box but different positions relative to the cue cards in each local compartment. (B) The behavioural testing paradigm on the fifth day, similar to the paradigm used in Experiments 1A, B and C: Trials 1-7 were identical to habituation in the first four days; and Trial 8 was the probe trial where the location of a single object was changed to render the environment visually symmetrical.

5.1.3.4 Probe trials

For the pilot experiments, the 10th trial of the experiment was a probe trial (Figure 5.2B). For the full paradigm, the probe trial was the 8th trial on Day 5. In this test trial, one of the objects was displaced to the opposite end of its compartment (i.e. from far from the landmark to near, or near to far). The object's position in relation to the landmark and the doorway were different, but the distance of the object from its nearest three walls remained the same (25cm). The other object in the pair was also handled to prevent differences in transferred cues, and the displaced and non-displaced objects were counterbalanced between animals. Thus, the relative object position in each compartment was visually symmetrical, leaving only the olfactory context providing a way to resolve the directional ambiguity of the landmarks. If the animal correctly encoded the olfactory context as well as the location of the object relative to the

landmark and door, this spatial change should initiate recognition of novelty and increased exploration of the displaced object.

Similarly to the habituation trials, the apparatus was rotated and the objects were interchanged randomly beforehand. The displacement of the objects was also counterbalanced across animals. As the scorer was not blinded to the displacement of objects, all trials were blindly re-scored post-hoc from a recorded video of the experimental session. The correlation in the two scorers' datasets was high, differing only by a few seconds on average. Where discrepancies were found, these trials were revisited by both scorers together post-hoc and a value was agreed.

5.1.3.5 Data Analysis

The raw object exploration data were analysed using one-way and repeated measures ANOVA, and the 2-tailed t-test.

To assess whether there was any difference in exploration between trials (or days for the full paradigm), one-way and repeated measures ANOVA were performed. 'To further characterise any differences in exploration and assess whether there was a learning trend either within an experimental day (the whole session for a pilot, or one day in the full paradigm) or across the full experiment (five experimental sessions, one per day), Jonckheere trend tests were performed (Jonckheere and Bower, 1967). For details see section 4.4.2.2.

Differential exploration of the displaced and non-displaced objects in the probe trial was assessed using a discrimination ratio and the 're-exploration score'. For details see section 4.4.2.2.

Effect size analysis was used to gauge whether the difference in re-exploration score between displaced and non-displaced objects was significant and meaningful. Effect size was reported using Cohen's *d* statistic: $d = 0.2$ is considered a 'small' effect size, $d = 0.5$ represents a medium effect size, and $d = 0.8$ a large effect size (Cohen, 1988).

5.2 Experiment 1A – Pilot 1

The present pilot experiment (the first in a series of three) aimed to replicate aforementioned preliminary results, obtained using familiar animals, in naïve animals (n = 6). These previous preliminary results indicated that animals preferentially explored the displaced object in the probe trial. The timeline of Experiment 1A is detailed below in Figure 5.4.

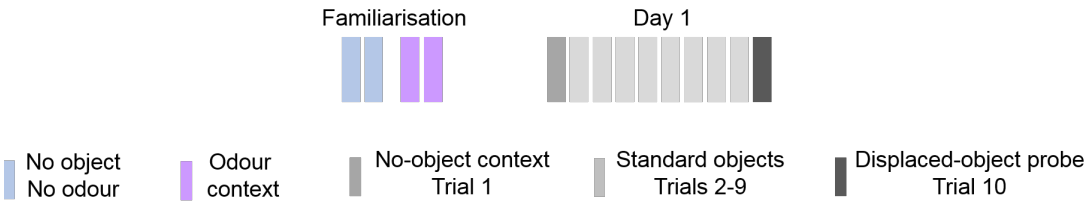


Figure 5.4: Timeline of experimental session in Experiment 1A (Pilot 1). Animals were first given two sets of familiarisation trials at a rate of two per day (two without odour or objects, and two with odours but no objects). Following this, the pilot experimental paradigm consisted of a single re-familiarisation trial with odours but no objects, 8 habituation trials with objects, and a probe trial where the objects were displaced to render the environment visually symmetrical.

5.2.1 Results – Pilot 1

Exploration time – decline in time across trials

As two odours were used, it was first necessary to prove that one odour did not bias either more or less object exploration. The results of André & Manahan-Vaughan (2013) suggest that rats may have found the lemon odour aversive, due to a significantly lower exploration of a lemon-scented compartment versus vanilla and almond. Per day the proportion of time animals spent exploring each compartment in the habituation phase (trials 2 to 9) varied but neither odour elicited a significant preference, as would be demonstrated by a greater proportion of exploration time (repeated measures ANOVA, [$F(1,10) = –.016$, $p = .902$]).

To see whether there was any trend in animal behaviour across the habituation trials (trials 2 to 9), a one-way ANOVA was performed. Trial 1 was a re-familiarisation session without objects, and so total object exploration was not calculated for this trial. The exploration over the habituation trials was not significantly different (one-way ANOVA, [$F(7,47)=.491$, $p = .835$]). To further categorize any behavioural trend (*i.e.* to see if learning occurred) over trials, the exploration times were also subjected to a directional Jonckheere trend test. The total exploration time trends towards a decrease from trial 2 to trial 9 in the habituation phase ($Z = -2.15$, $p = .031$). The pattern of object exploration is non-uniform, with animals occasionally demonstrating increased or decreased interest from trial to trial; this reflects the fact that only spontaneous animal behaviour is being assessed.

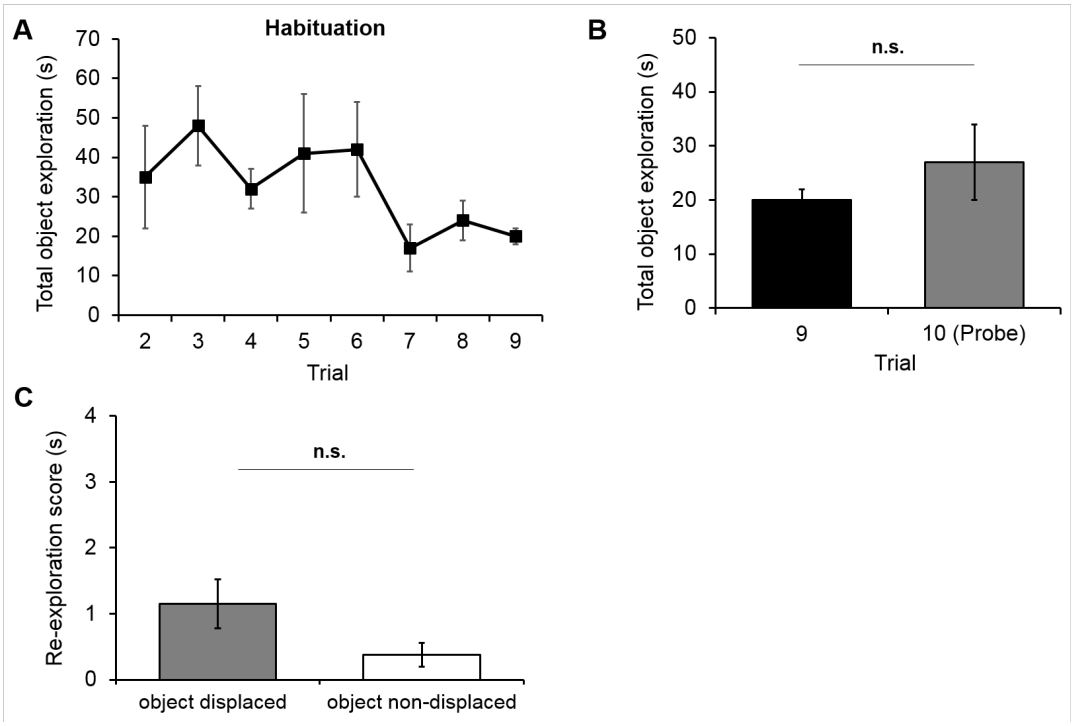


Figure 5.5: Performance of animals in the habituation and probe trials of the pilot experiment (Experiment 1A). (A) Pooled total object exploration over the habituation trials (2-9) shows a decreasing trend ($p < .05$). (B) Comparison of total object exploration between the final habituation trial 9 and probe trial 10. There was no significant difference in exploration at test, indicating that the animals were not affected by the novel displacement. There was also no significant difference in object exploration using the re-exploration score (C), evaluating time spent interacting with each of the displaced and non-displaced objects.

Probe trial does not induce increased exploration of novel displaced object

To first see whether there was any difference between the animals' performance in habituation trials and the probe trial, an initial comparison was made of total object exploration in the final habituation trial (trial 9) and the probe trial 10. The non-significant increase between trials 9 and 10 (Figure 5.5B) indicates that even though object exploration was different in the probe trial, there was not a significant increase from baseline levels of exploration to indicate a substantial novelty effect and recognition of object displacement (paired t-test (2-tailed), $t(5) = 0.153$, $p = .884$). This insignificance is in conflict with the results from the preliminary results.

For detailed analysis of the rats' behaviour in the probe trial and test day, the objects were categorised according to whether they were displaced or non-displaced. A D2 discrimination ratio was calculated to assess if rats were discriminating between the displaced and non-displaced objects (Appendix 2A). The discrimination ratio of trials 2-10 showed that the discrimination did not differ over the experiment (one-way ANOVA,

[$F(8,45) = 0.635$, $p = .744$]) and that the objects were not treated differently following displacement of one object to a novel position.

As the discrimination ratio is prone to noise created by small exploration values, a novel 're-exploration score' was developed to investigate the rats' discrimination between the displaced and non-displaced object at test: this score compares the exploration of each object (displaced or non-displaced) compared to the baseline exploration of that object throughout the prior habituation trials (Van Cauter et al., 2013). Figure 5.5C shows no significant difference in the re-exploration score between displaced and non-displaced object (paired t-test (2-tailed), $t(5) = 2.05$, $p = .096$), such that there was no increase of exploration in favour of the displaced object in the probe trial.

In summary, even though there was a decreasing trend in exploration time across trials, suggesting learning, there was no significant difference in either the amount of object exploration between trial 9 and probe trial 10 and similarly no significant difference in exploration of the displaced object compared to the non-displaced. As there was no increase in overall exploration in the probe trial, this suggests that the animals were unable to recognise on the whole that there was a change in the location-odour configuration in the probe trial. In addition to this there was no differential exploration, suggesting that they were also unable to recognise which object had been displaced.

As these results were in conflict with previous preliminary results from familiar animals, the animals used in the present experiment were subjected to a second round of the paradigm (Experiment 1B) to assess the amount of familiarisation necessary to see an effect in the probe trial.

5.3 Experiment 1B – Pilot 2

In the present pilot experiment (the second in a series of three), the same subjects were used as those used for Experiment 1A. These animals already had the following level of experience with the two-compartment apparatus: 2x 10-minute familiarisation sessions without odour and without objects, 2x 10-minute familiarisation sessions with odour but without objects, and a full experimental session of ten 4-minute trials (Figure 5.6).

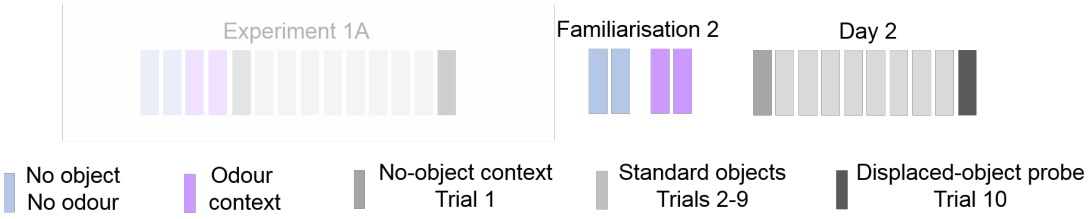


Figure 5.6: Timeline of experimental session in Experiment 1B (Pilot 2). Animals in Experiment 1B had already experienced the familiarisation, habituation and probe trials of Experiment 1A. Animals were then given another two sets of familiarisation trials at a rate of two per day (two without odour or objects, and two with odours but no objects). Following this, the pilot experimental paradigm again consisted of a single re-familiarisation trial with odours but no objects, 8 habituation trials with objects, and a probe trial where the objects were displaced to render the environment visually symmetrical.

5.3.1 Results – Pilot 2

Exploration time – decline in time across trials

The proportion of time animals spent exploring each compartment (lemon/vanilla) was analysed in habituation trials to determine whether either odour induced a significant preference, as determined by bias in exploration time. Similarly to the first pilot, there was no influence of odour on object exploration time (repeated measures ANOVA, [$F(1,10) = 1.88$, $p = .200$]).

Again, trial 1 was a re-familiarisation session without objects thus total object exploration was not calculated for this trial. Although the exploration over habituation trials did not significantly differ (one-way ANOVA, [$F(7,47)=1.367$, $p = .246$]), the raw total exploration time decreased over the habituation trials (trials 2 – 9) and there a decreasing trend which indicated that learning occurred (Jonckheere trend test, $Z = -2.84$, p (2-tailed) = .004; Figure 5.7A).

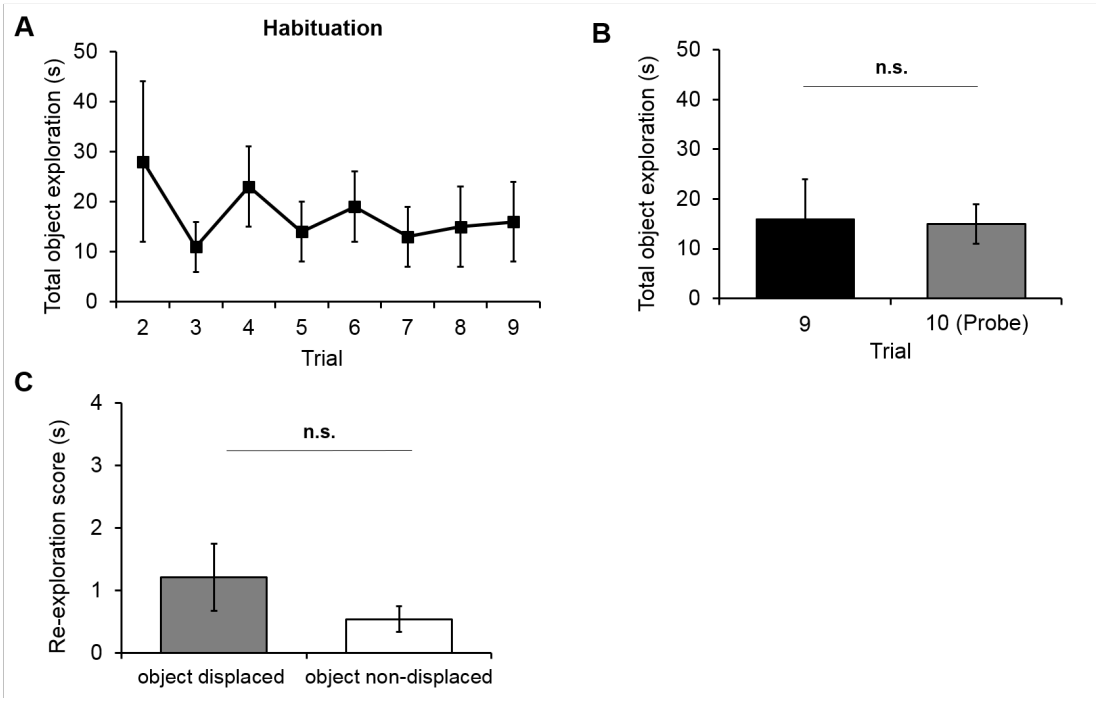


Figure 5.7: Performance of animals in the habituation and probe trials of the second pilot experiment (Experiment 1B). (A) Pooled total object exploration over the habituation trials (2-9) shows a decreasing trend ($p < .01$) (B) Comparison of total object exploration between the final habituation trial 9 and probe trial 10. There was no significant difference in exploration at test, indicating that the animals were not affected by the novel displacement. There was also no significant difference in object exploration when evaluating time spent interacting with each of the displaced and non-displaced objects; shown by the re-exploration score (C).

Probe trial does not induce increased exploration of novel displaced object

To assess whether there was an overall difference between the animals' performance in habituation trials and the probe trial, an initial comparison was made of total object exploration in the final habituation trial (trial 9) and the probe trial 10. There was a negligible and insignificant difference between trials 9 and 10 (paired t-test (2-tailed), $t(5) = 1.31$, $p = .247$; Figure 5.7B), indicating that on average there was no change in animals' exploration of the objects in the probe trial. Thus, there was still no significant increase from baseline levels of exploration to indicate a substantial effect of novelty and recognition of object displacement.

For detailed analysis of the rats' behaviour in the probe trial and test day, the objects were categorised according to whether they were displaced or non-displaced. Examination of the D2 discrimination ratio, showing whether the animal spent unequal amounts of time exploring one object or another, showed that the discrimination did not differ over the experiment (one-way ANOVA, [$F(8,45) = 1.069$, $p = .402$; Appendix 2B). Examination of the re-exploration score, similar to the discrimination index but instead showing whether the animal spent compared to baseline, also showed no significant

difference in exploration time of the displaced object in the test trial (paired t-test (2-tailed), $t(5) = 1.01$, $p = .358$]; Figure 5.7C).

In summary, even though there was a decreasing trend in total exploration time across trials, suggesting that rats learned about the environment, there was still no significant difference in amount of exploration between trial 9 and probe trial 10, and similarly no significant difference in exploration of the displaced object compared to the non-displaced when evaluated using the discrimination ratio and re-exploration score. As in Experiment 1A (Pilot 1), the animals were still unable to detect a change in the global location-odour configuration or recognise which object had been displaced. These animals were thus subjected to a third and final instance of the same pilot paradigm in Experiment 1C (Pilot 3) with novel textures added to the objects.

5.4 Experiment 1C – Pilot 3

In the present pilot experiment (the last in a series of three), the same subjects were used as those used for Experiment 1A and 1B. These animals already had the following level of experience with the two-compartment apparatus: 4x 10-minute familiarisation sessions without odour and without objects, 4x 10-minute familiarisation sessions with odour but without objects, and two full experimental sessions of ten 4-minute trials (Figure 5.8).

Figure 5.8: Timeline of experimental session in Experiment 1C (Pilot 3). Animals in Experiment 1C had already experienced the familiarisation, habituation and probe trials of Experiments 1A and 1B. Animals were then given another two sets of familiarisation trials at a rate of two per day (two without odour or objects, and two with odours but no objects). Following this, the pilot experimental paradigm again consisted of a single re-familiarisation trial with odours but no objects, 8 habituation trials with objects, and a probe trial where the objects were displaced to render the environment visually symmetrical.

5.4.1 Results – Pilot 3

Exploration time – decline across trials

As before, the proportion of time animals spent exploring each compartment (lemon/vanilla) was analysed in habituation trials to determine whether either odour induced a significant preference, as determined by bias in exploration time. Similarly to the first and second pilots, there was no influence of odour on object exploration time (repeated measures ANOVA, [$F(1,10) = .798$, $p = .393$]).

Trial 1 was a re-familiarisation session without objects, so total object exploration was not calculated for this trial. Figure 5.9A shows a clear decrease in raw exploration times over the habituation phase (trials 2 – 9), with this exploration significantly different between trials (one-way ANOVA, [$F(7,47) = 3.906$, $p = .002$]). A directional trend test confirmed this decreasing trend and indicated that learning occurred (Jonckheere trend test, $Z = -4.10$, p (2-tailed) < 0.001).

Object displacement in the probe trial induces increased exploration of novel displaced object

Two phases were used to assess whether there was an overall difference between the animal's performance in habituation trials and the probe trial. First, an initial comparison was made of total object exploration in the final habituation trial (trial 9)

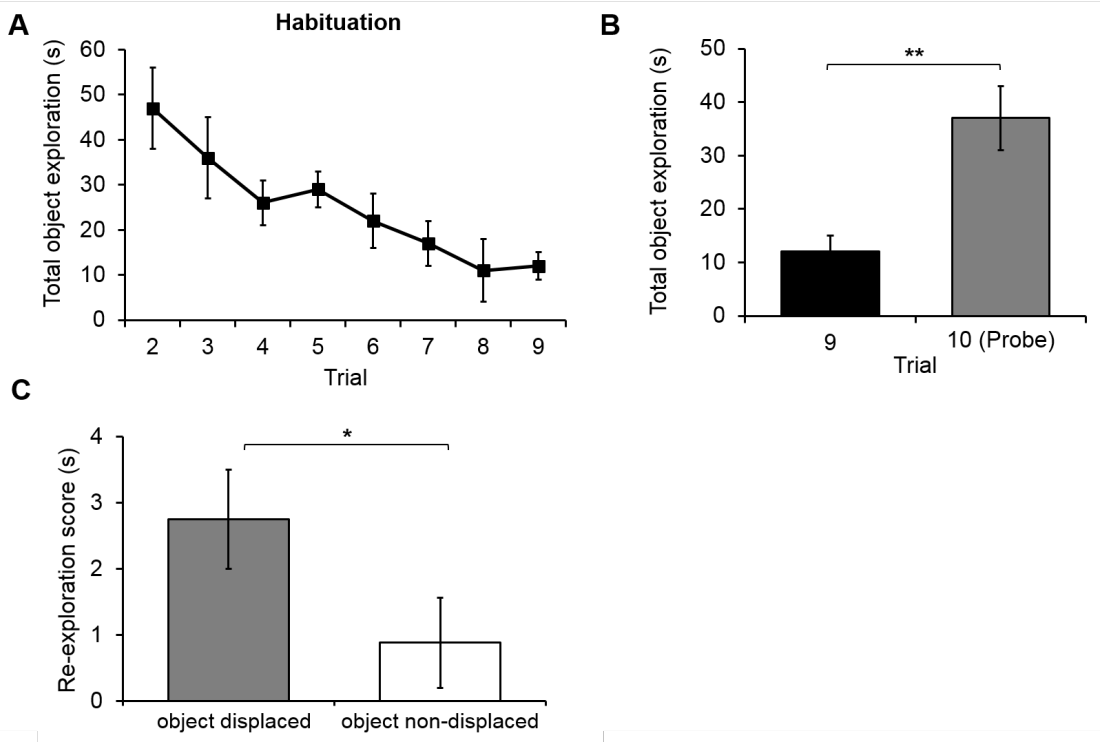


Figure 5.9: Performance of animals in the habituation and probe trials of the third pilot experiment. (A) Pooled total object exploration over the habituation trials (2-9) shows a clear decreasing trend ($p < .001$). (B) Comparison of total object exploration between the final habituation trial 9 and probe trial 10. There was a significant difference in exploration at test ($p < .01$), indicating that the animals were influenced by the novel displacement. There was significant difference in object exploration when using the re-exploration score to evaluate time spent interacting with each of the displaced and non-displaced objects ($p < .05$) (C). This result shows that the overall object exploration increased in the probe trial, and the animals significantly favoured the displaced object.

and the probe trial (trial 10). There was a significant difference between trials 9 and 10 (paired t-test (2-tailed), $t(5) = -4.35$, $p = 0.007$; Figure 5.9B), indicating that, on average, the animals explored the objects significantly more in the probe trial.

Thus, compared to habituation, there was a significant increase from baseline levels of exploration to indicate a substantial novelty effect and recognition of object displacement.

Second, the objects were categorised according to whether they were displaced or non-displaced, and the displacement ratio and re-exploration score were examined. These tests examined whether the animal spent unequal amounts of time exploring one object or another. Raw exploration values comparing displaced and non-displaced objects show that on average, 74% of exploration in the probe trial was of the displaced object (average raw exploration values: displaced, $M = 27.7$s, $SE = 6.34$s; non-displaced, $M = 9.67$s, $SE = 3.89$s). While the discrimination ratio showed no significant change in object discrimination over the experiment (one-way ANOVA, [$F(8,45) = 1.015$, $p = .438$]; Appendix 2C), the re-exploration score indicated a significant difference between the displaced and non-displaced objects (average re-exploration

scores: displaced, M = 2.75s, SE = 0.75s; non-displaced, M = 0.74s, SE = 0.69s) (paired t-test (2-tailed), $t(5) = 2.73$, $p = .041$; Figure 5.9C), supporting the raw data in that there was an increase in exploration in favour of the displaced object in the probe trial.

In summary, similarly to the pilot experiments 1A and 1B, there was a clear decreasing trend in total exploration time across trials indicating that learning occurred. However, additionally, the results of the present pilot experiment show both a significant difference in the total amount of object exploration between trial 9 and probe trial 10 as well as a significant difference in exploration of the displaced object compared to the non-displaced in the probe trial when evaluated with the re-exploration score. These results suggest that, in contrast to the earlier experiments in this series, the animals were now able to recognise that there was a change in the global 'location-odour' configuration, and correctly distinguish which object had been displaced.

The results of Experiments 1A, 1B, and 1C indicate that the animals evaluated in the preliminary study were considerably more familiar with the environment than previously recognised, and that extensive familiarisation with the two-compartment environment was necessary to replicate the results with naïve animals. To this end, we adjusted the experimental paradigm to include five consecutive days of habituation to the two-context environment (of eight trials per day), with the probe trial replacing the final habituation trial on the fifth day.

5.5 Experiment 1D – Full paradigm

The timeline for the five-day full paradigm is shown below in Figure 5.10; for details on experimental testing procedures refer to sections 5.1.3.3 and 5.1.3.4. Animals used here were different to those in Experiments 1A, 1B and 1C, and were naïve to the testing environment (n = 10).

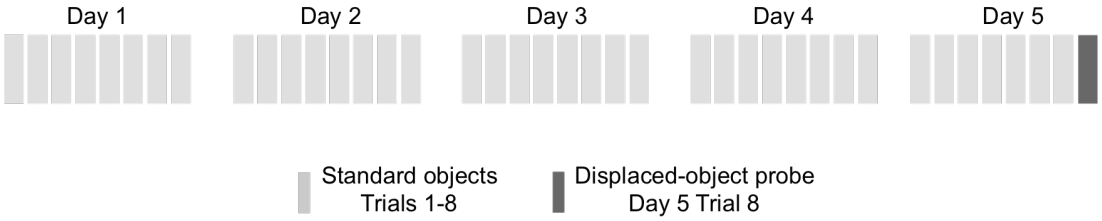


Figure 5.10: Timeline of the five experimental days in Experiment 1D (Full paradigm). Animals were given four days of habituation to the 2-compartment box with both odours and objects, at a rate of eight trials per day. Following this, they were subjected to a final day of seven habituation trials and a final probe trial as the eighth. In the probe trial, a single object was displaced to render the environment visually symmetrical.

5.5.1 Results – Final paradigm

Exploration of objects – decline across and within days

Over each session in a given day, the proportion of time animals spent exploring each compartment varied but neither odour influenced a significant preference (Figure 5.11), which would be demonstrated by a greater proportion of exploration time (repeated measures ANOVA, [$F(1,18) = .021$, $p = .887$]).

As exploration time did not vary significantly in response to odour, the exploration times of the vanilla and lemon objects could be pooled within each trial to obtain a total exploration time measure. Total exploration during the full habituation phase (excluding Day 5 Trial 8) was significantly different between days (repeated measures ANOVA, trial x day, [$F(9,81) = 6.04$, $p < .001$]; Figure 5.12A).

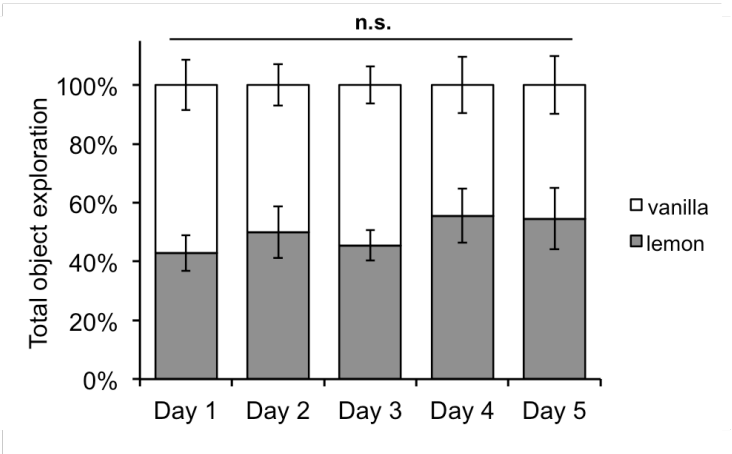


Figure 5.11: Olfactory context has no effect on exploration or habituation over days. The total object exploration time over five days (Monday – Friday), represented by the proportion of lemon and vanilla object exploration (mean±SEM), does not significantly differ between olfactory contexts.

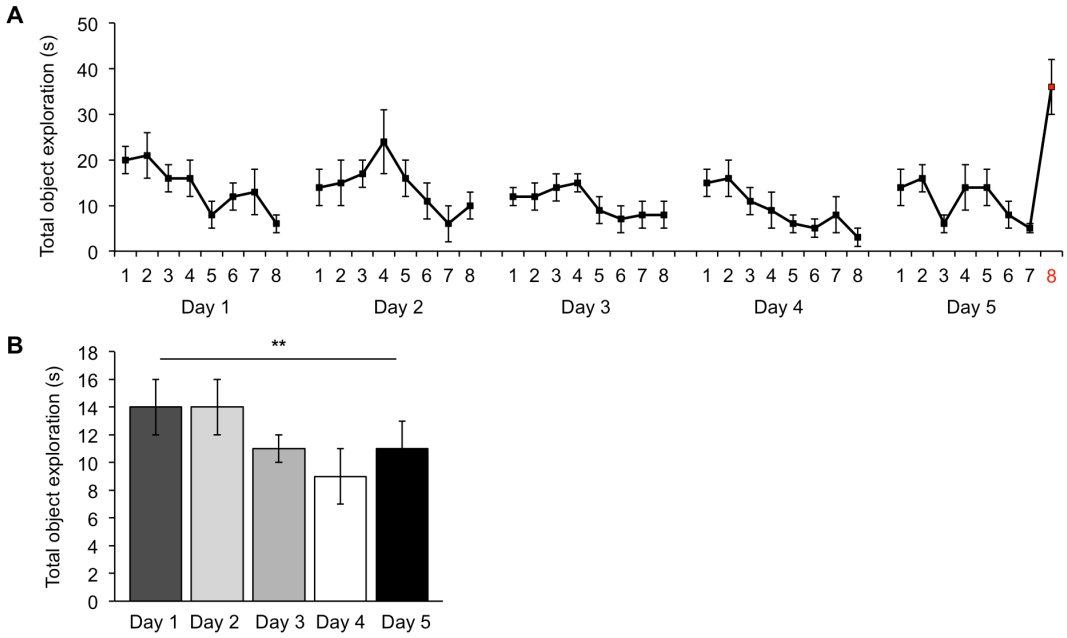


Figure 5.12: Object exploration over days decreases but the probe trial elicits a large increase in exploration. (A) Pooled total object exploration over each of the 8 trials per day demonstrated a negative trend over the habituation trials of the five days ($p < .0001$), and a large increase in exploration time in the probe trial (trial 8 on Friday). There was also a significant negative trend over habituation trials within each day (Day 1, $p < .001$; Day 2, $p < .05$; Day 3, $p < .01$; Day 4, $p < .001$; Day 5, $p < .05$). Probe trial is indicated by a red marker and red text. (B) There is a significant difference in the average exploration in habituation trials between days, indicating learning [$p < .01$].

However, the repeated measures ANOVA does not assess the direction of the difference itself. To confirm the direction of the trend in animal behaviour (*i.e.* to assess whether learning occurred) over trials within the same day or across days, the exploration times were then subjected to a Jonckheere trend test. There was a significant negative trend in object exploration within each day, indicating that learning occurred during the course of a single experimental session (Day 1, $Z = -3.58$, $p = .0003$; Day 2, $Z = -2.56$, $p = .0104$; Day 3, $Z = -2.68$, $p = .007$; Day 4, $Z = -3.99$, $p = .0001$; Day 5, $Z = -2.16$, $p = .0307$). Given our results in the early pilot experiments, the negative trend on day 1 is surprising but this may be in part due to an increase in numbers of animals, making the average more robust. There was also a significant negative trend in object exploration over the full experiment (habituation trials between days, $Z = -3.05$, $p = .002$), suggesting that the animals became familiar with the location-odour context in the two-compartment apparatus over the course of the five days leading up to the probe trial (Figure 5.12A).

In an analysis of average total object exploration between days of habituation trials (trials 1-8 on Monday, Tuesday, Wednesday and Thursday, and trials 1-7 on Friday) (Figure 5.12B), a repeated measures ANOVA found a significant difference between days [$F(4,36) = 4.18$, $p = .007$]. This supports the suggestion that learning occurred

over the five habituation days, leading to less object exploration between days. It should be noted that an effect of partial dishabituation at the beginning of each day can be seen in Figure 5.12A.

Probe trial induced re-exploration of the displaced object

As in the pilot experiments, determination of whether the rat's behaviour at test was different to habituation was done in two phases. First, an initial comparison was made of total object exploration in habituation trials and the probe trial on Day 5. To create a similar baseline as used in the re-exploration score, an average of the object exploration in habituation trials on Day 5 was calculated and then compared to the probe trial. Total object exploration was significantly higher at test (probe trial) compared to habituation (trials 1-7) (average exploration values: baseline, M = 11.2s, SE = 1.31s; probe trial, M = 36.0s, SE = 6.24s) (paired t-test, $t(9) = -4.30$, $p = .002$; Figure 5.13A). This indicates that the animals had a renewed interest in the objects in the probe trial.

Second, the difference in exploration of the displaced and non-displaced objects was examined. Raw exploration data shows that, on average, 81.7% of the total exploration in the probe trial was of the displaced object (average exploration values: displaced, M = 29.4s, SE = 4.60s; non-displaced, M = 6.60s, SE = 2.89s). Examination of the discrimination ratio did not show a significant difference in object discrimination over the experiment (one-way ANOVA, [$F(7,79) = 2.117$, $p = .052$; Appendix 3A) but it must be taken into consideration that this measure is prone to noise generated by small exploration values. The re-exploration score, showing whether the animal spent unequal amounts of time exploring one object or another relative to the trial baseline, supports the raw data and demonstrates a significant difference in exploration of the displaced and non-displaced objects (average re-exploration scores: displaced, M = 7.14s, SE = 2.02s; non-displaced, M = 1.01s, SE = 0.41s) (paired t-test, $t(9) = 2.82$, $p = .020$; Figure 5.13B). Effect size analysis between the re-exploration score for each object category revealed that this difference was significant and meaningful (raw difference = 6.13s, Cohen's d = 1.33).

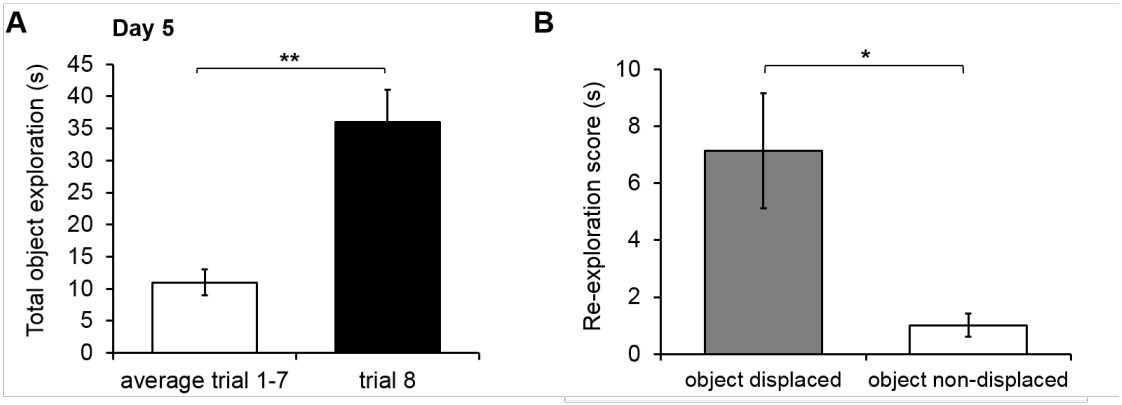


Figure 5.13: Probe trial exploration is significantly different from habituation. (A) Object exploration in the probe trial (Day 5, trial 8) is significantly higher, at the $p < 0.01$ level, than the average of habituation trials 1-7 on the same day. (B) Examination of the re-exploration score for the probe trial shows that the animals significantly favoured exploration of the displaced object over the non-displaced ($p < 0.05$).

Taken together, these results from raw exploration time and the re-exploration score indicate that the animals could successfully discriminate between the locations of the two objects and actively explored the displaced object for a longer period of time in the test trial.

To summarise, there was a decreasing trend in total exploration time across trials in each day, suggesting that rats successfully learned and encoded the location of the objects relative to the landmarks and odour context in the habituation phase. At test, the animals explored the displaced objects significantly longer than non-displaced objects when normalised to baseline, as well as showing a distinct preference for the displaced object at a greater than chance level.

5.6 Discussion

In this study, we developed a novel spontaneous recognition task to show that rats are able to associate objects to particular locations in an environment where these locations can only be uniquely identified by combining landmark and odour cues. While similar experiments have assessed the ability of rats to perform configural processing (Eacott and Norman, 2004; Norman and Eacott, 2005), this is the first to require the use of contextual information to disambiguate directional information within a task.

The 'location-odour' task

While based upon the 'object-place-context' or 'what-where-which' task of Eacott and Norman (2004), the task described here differs in that the identity of the objects themselves are consistently identical and thus not important. With this in mind, this 'location-odour' task can be better aligned to the 'context-place' or 'which-where' task of Easton et al. (2010) where subjects were only required to associate a place with the context it is located in.

The 'location-odour' task takes fundamental principles from the spontaneous recognition task, exploiting rats' tendency to explore novel aspects of their environment (Ennaceur and Delacour, 1988). The findings from the final paradigm (Experiment 1D) showed that when one object was displaced at test, creating a visually symmetrical environment, the rats showed a clear preference (demonstrated by a significant increase in exploration time and re-exploration score) for objects in a novel location-odour configuration over an object in a familiar location-odour configuration. This provides strong evidence that the two olfactory contexts can provide orienting cues that are just as salient as visual information, and that behaving animals can integrate this context to encode the location of objects and resolve directional ambiguity.

With the preliminary experiments in mind, where animals familiar with the context box demonstrated a clear discrimination of the displaced and non-displaced objects in a single day paradigm, the three pilot experiments aimed to ascertain the level of familiarity needed for naïve animals to demonstrate the same level of discrimination. Between the first and second pilot, animals were only exposed to additional experience with the context box environment; but between the second and third pilot experiment, the same animals were exposed to even further experience with the environment and a texture-enriched set of objects. Thus, the discrimination results from the third pilot could have been driven by either novelty (as the objects could be treated as new) or extended training. As it would not have been possible to disentangle these two

changes without additional pilot experiments in naïve animals, both changes were carried over into the final paradigm.

This type of task with free exploration demonstrates an animal's natural behaviour rather than any trained behaviour or behaviour resulting from aversion or demand. As the compartments were distinguishable by the locations of the objects relative to landmarks during habituation, it is assumed that there is no demand on the animal to pay attention to the local odour cues for navigation but as per latent learning (Tolman, 1948) these should be processed regardless. Once the available cues were degraded to odour alone, the rat was able to use the specific odour contexts to recall the location-odour configuration successfully – this demonstrates that rats do indeed passively encode olfactory information without demand, and that olfactory information can be used as an orienting source especially under directional ambiguity. This fits well with neural data that found the hippocampus can substitute for missing visual information by using olfactory spatial context information to facilitate synaptic plasticity and enable spatial information encoding (André and Manahan-Vaughan, 2013).

In addition to indicating increasing familiarity, the learning trend over the initial days of the full paradigm may reflect increasing stability and depth of the animals' spatial representation. Barry et al. (2007) found that the scale of grid cells recorded in the mEC expands coincidentally with the animal being introduced to a novel environment, but that this expansion then returns to baseline with three or four days of repeated experience. This suggests that spatial representations of environments become more stable with increasing experience, and could support the reasoning that naïve animals in the pilot experiments did not strongly react to the spatial displacement because their representation of the location-odour configuration was incomplete.

Although learning was noted within days, between days there was a noticeable effect that habituation from the previous day partially reversed after the 24-hour rest period. From observation of the animals' behaviour, this effect may not have been due to a reversal of learning but more to do with dishabituation or novelty detection after elapsed time. Given that there is no goal-directed behaviour or reward in this task, all learning being done is latent (Tolman, 1948) and thus it is more likely that the animals just initially explored more due to a curiosity of being in different surroundings (despite them being familiar).

Other interpretations

The contexts driven purely by odour in this experiment are different to conventional descriptions of context. This discussion so far has assumed that the distinct odours in

each compartment of the apparatus would create two distinct contexts, but there is a possibility that the box could be treated as a single context with the odours acting as orienting cues similar to the cue card landmarks. Recording of grid cells in a two-compartment connected environment (as in Skaggs and McNaughton (1998) (Figure 3.3A) showed that there was an experience dependent change in the grid cell representation of these spaces: initially, the grid firing patterns were dominated by local environmental cues and displayed replicated patterns in the two compartments; but with experience, grid firing patterns formed a single continuous representation that spanned the environment as a whole (Carpenter et al 2015). This transition suggests that, within a 2 week period (15 sessions at a rate of 1 per day), grid cells adjust their firing to produce a globally coherent representation of the space as a whole rather than its composite compartments. In experiment 1D of this thesis, the animals were trained for only 5 sessions at a rate of one per day; thus with the timescale set by Carpenter et al. (2015) it would take an additional 10 sessions for grid cells to reach a single continuous representation of this apparatus so at the point of test, the compartments would still have been treated as separate.

It must also be noted that as no control experiment was carried out in the two-compartment context apparatus without the use of any odours, it remains unproven that odour acts as the disambiguating cue. However, it is highly unlikely that the animals could resolve the spatial displacement at test without odours given the visual symmetry of the local environment from the point of view of the animal and the absence of any global cues or experimenter-influenced processes (i.e. initially placing the rat consistently in one particular compartment) (Rosenthal and Fode, 2007).

In summary, the present study shows that rats can use olfactory context cues to create complex conjunctions of stimuli (in this case, location-odour) and resolve directional ambiguity of a visually symmetrical environment. A novel aspect of this experiment was that animals had *two* methods of discriminating context: by odour or by directional orientation, where the orientation was informed by the odour. In other words, the influence of odour on the compartment discrimination could have been direct, or indirect via the head direction system. The next two experiments explore this issue. Experiment 2 records place cells to determine whether they remapped or repeated their fields between compartments, and if they repeated them, whether the fields reversed orientation between compartments. Experiment 3 manipulated the head direction system directly, by inactivating the anterior thalamic nuclei, to see whether rats could make the discrimination under a disruption of the head direction signal.

Chapter 6 - Experiment 2

Can place cells resolve conditions of directional ambiguity?

As previously discussed, place cells display localised maximal firing in a particular environment and are largely silent elsewhere (O'Keefe and Dostrovsky, 1971). A population of place cells can represent the entire surface area of an environment, as different place cells have place fields in different spatial locations. Along with input from neurons such as head direction, grid and boundary cells, place cells are thought to form the core of a neural 'cognitive map' or an internal representation of the animal's external world (O'Keefe and Nadel, 1978; Tolman, 1948). The previous experiment found that animals could solve the directional ambiguity presented by visually symmetrical two-compartment apparatus using odour context information, and raised the question of how this resolution was achieved. This experiment explores this question by recording place cells to find out whether this ambiguity can be resolved on a neuronal level.

Studies of place cells in similar spaces have revealed that their spatial representation is prone to repetition (Grieves et al., 2016; Spiers et al., 2015), suggesting that place cells are poor at dealing with visual symmetry. However, introduction of directional information as another distinguishing cue led to a decrease in the amount of repetition (Fuhs et al., 2005; Grieves et al., 2016), such that the place cell system could realise the spaces as separate. Jacob et al. (2016) conducted an investigation into HD cells in the same two-compartment context box as the present study, finding that most directional neurons across the HD network (ADN (see Appendix I), PoS and RSC) could use the odour context to create a global representation of direction across the whole environment, while a small population of neurons seemingly anchored purely to the local landmarks and 'flipped' preferred firing direction 180° between compartments.

Experiment 2 draws on the knowledge that the head direction system provides critical information to hippocampal place cells about the animal's orientation. Place cells were recorded to assess whether they too present with fields defined by local visual cues between compartments (*i.e.* their fields would be 'flipped' between compartments) or not. The absence of locally controlled place field activity would suggest that the global/local encoding disparity resulting in the two head direction signals is resolved by the time the signal reaches the hippocampus. It would also suggest that place cells can use odour context, likely through head direction information, to resolve the directional ambiguity of the environment and realise the two compartments as separate.

6.1 Methods

6.1.1 Animals

Three male Lister hooded rats (Charles River, UK; weighing at least 350g at implantation) participated in this study. After each pass through the experimental protocol, the tetrodes were advanced in order to find new cells. If new, unique, place cells were located then the experiment was repeated.

6.1.2 Experimental apparatus

The two-compartment context box (see Figure 4.2) was placed in exactly the same position within the laboratory room from trial to trial, regardless of orientation, and surrounded by floor length black curtaining for all trials. The curtaining was in place to make sure that external cues outside the context box itself (*e.g.* features of the testing room like recording equipment and shelves) were out of view of the animal during testing. Six circularly arranged ceiling lights lighted the box, and a non-tuned radio was fixed centrally above the box producing a background noise >70dB to mask uncontrolled directional sounds. The experimenter constantly entered/exited the curtained environment from different places to avoid becoming a salient cue for the animal.

6.1.3 Experimental protocol

If spatially modulated cells (place or head direction cells) were found during a screening session, then the cells were recorded in the experimental protocol. This protocol was the same as that of Jacob et al. (2016*)*. Each experimental session comprised five trials, an example is shown in Figure 6.1: one baseline (15 minutes), one rotation (15 minutes), compartment 1 only (10 minutes), compartment 2 only (10 minutes), and a second baseline (20 minutes). The last trial is a repeat of the first baseline trial to ensure inter-trial stability of spatial cells, and the rotation between the

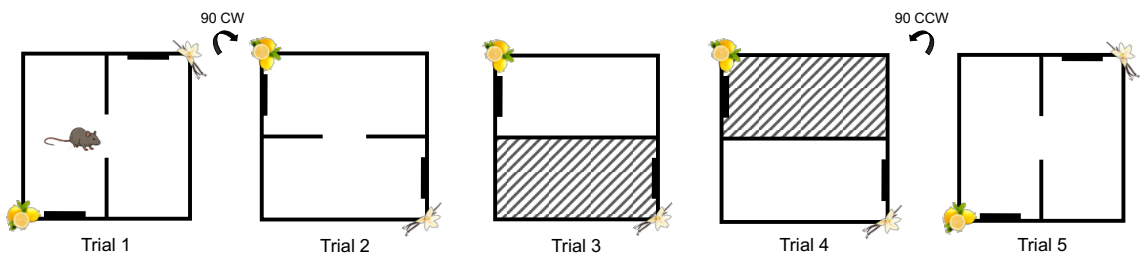


Figure 6.1: Experimental protocol for electrophysiological recordings. The two-compartment box comprised two visually identical but oppositely oriented compartments, one scented with lemon and the other with vanilla. The animal was able to move freely between the two compartments in sessions 1, 2 and 5, but was constrained to a particular compartment in trials 3 and 4. The apparatus was subject to randomly selected rotations (0, -90, 180, +90 degrees) between trials. Trial 5 served as a baseline, in the same orientation as trial 1.

first and second trials was to ensure landmark control of cells (cells are following the intramaze cues rather than any external features). The length of the trials was judged on a trial-by-trial basis for the single compartments and final trial so sufficient sampling was achieved. In between trials, animals were placed in a holding box for approximately 3-5 minutes, to allow for cleaning/changing of the recording environment. During each trial, rice was scattered randomly into the environment to encourage active foraging of the animal and ensure even spatial sampling; normal unflavoured rice was always scattered in the lemon odour box, and vanilla flavoured rice was always scattered in the vanilla odour box to further reinforce the context cues.

6.2 Results

6.2.1 Inter-trial stability threshold

A total of 500 units, including 123 unique place cells (spatial activity identified by eye, with peak firing rate above 1 Hz) and 4 unique head direction cells, were recorded from three animals. Of these 123 place cells, only one cell failed to pass the 50% coverage criterion described in section 4.4.3.4. Thus, a total of 122 place cells from three animals were accepted into further analysis for this experiment (number of place cells per animal: R590, n = 68; R608, n = 15; R609, n = 39). To assess the stability of place cell representation over the whole experimental session, a spatial correlation was run between the baseline of trial 1 and the baseline of trial 5 (for experimental protocol, see Figure 6.1): 107 cells passed the remapping threshold of 0.3, *i.e.* their representations remained stable over the experimental session (mean correlation coefficient, r = 0.580); while 15 cells were classified as unstable (mean correlation coefficient, r = 0.140).

6.2.2 Heterogeneous place cell representation in the two-compartment box

Having defined populations of cells for analysis based on stability across the entire experimental session, analyses were separately run on the 107 place cells passing the remapping threshold (termed ‘stable cells’) and the 15 non-passing cells (termed ‘unstable cells’).

From the current literature, three hypotheses were formed to describe likely place cell behaviour in this apparatus:

1. Place cells would be able to utilise the contextual cues to disambiguate the two compartments, and would display remapped fields across the apparatus to represent each compartment separately.
2. Place cells would recognise the differing context, but display duplicated fields.
3. Place cells would not be able to use contextual cues and rely on visual landmarks only resulting in fields that were ‘flipped’ between the compartments, aligned to the door axis.

Recorded place cells displayed heterogeneous place cell representation in the two-compartment context apparatus: one type of place cell displayed remapped fields between the two odours (85.0% of stable recorded cells, 100% of unstable recorded cells; Figure 6.5A; Table 2); while another type displayed duplicated fields across the two odours (15.0% of stable recorded cells, 0% of unstable recorded cells; Figure 6.5B;

Table 2). Examples of these types of cells are shown in Figure 6.2 and 6.5. The overwhelming percentage of remapped fields compared to duplicated suggests that the hippocampus can use odour context to disambiguate environments, and that this is primarily represented through clear remapping but that there is evidence for multi-layer context integration given the existence of the second population. The types of representation do not appear to be tied to cells that are segregated in physical space, as both types of cell were co-recorded. No instances of 'flipped' fields were observed. A full description of all recorded place cells and their types can be seen in Table 2.

To test whether the presence of remapped or duplicated fields were affected by the animals' experience with the context box, recordings were stratified by exposure time to the apparatus. In line with Barry et al. (2012), early experience was defined as ≤ 3 exposures to the apparatus while late experience was defined as 4 or more exposures. Experience did not affect the number of place cells recorded with remapped or duplicated field place cells, as both categories were equally represented in both the early and late timeframes (simple statistical test (Pocock, 2006), remapped: $z = 0.388$, $p > 0.05$, duplicated: $z = -0.5$, $p > 0.05$) (Appendix 5).

	Animal				
	R590	R608	R609	**Total**	**%**
Stable					
Remapped	46	12	33	91	85.0
Duplicated	11	1	4	16	15.0
'Flipped'	0	0	0	0	0
Unstable					
Remapped	11	2	2	15	100
Duplicated	0	0	0	0	0
'Flipped'	0	0	0	0	0
Total	68	15	39	**122**	

Table 2: Heterogeneous place cell representation in the two-compartment box. The number and % of place cells in each of the three categories (remapped, duplicated, 'flipped') for the stable and unstable populations. Each instance of a cell in the table represents a unique place cell (n=122 in total).

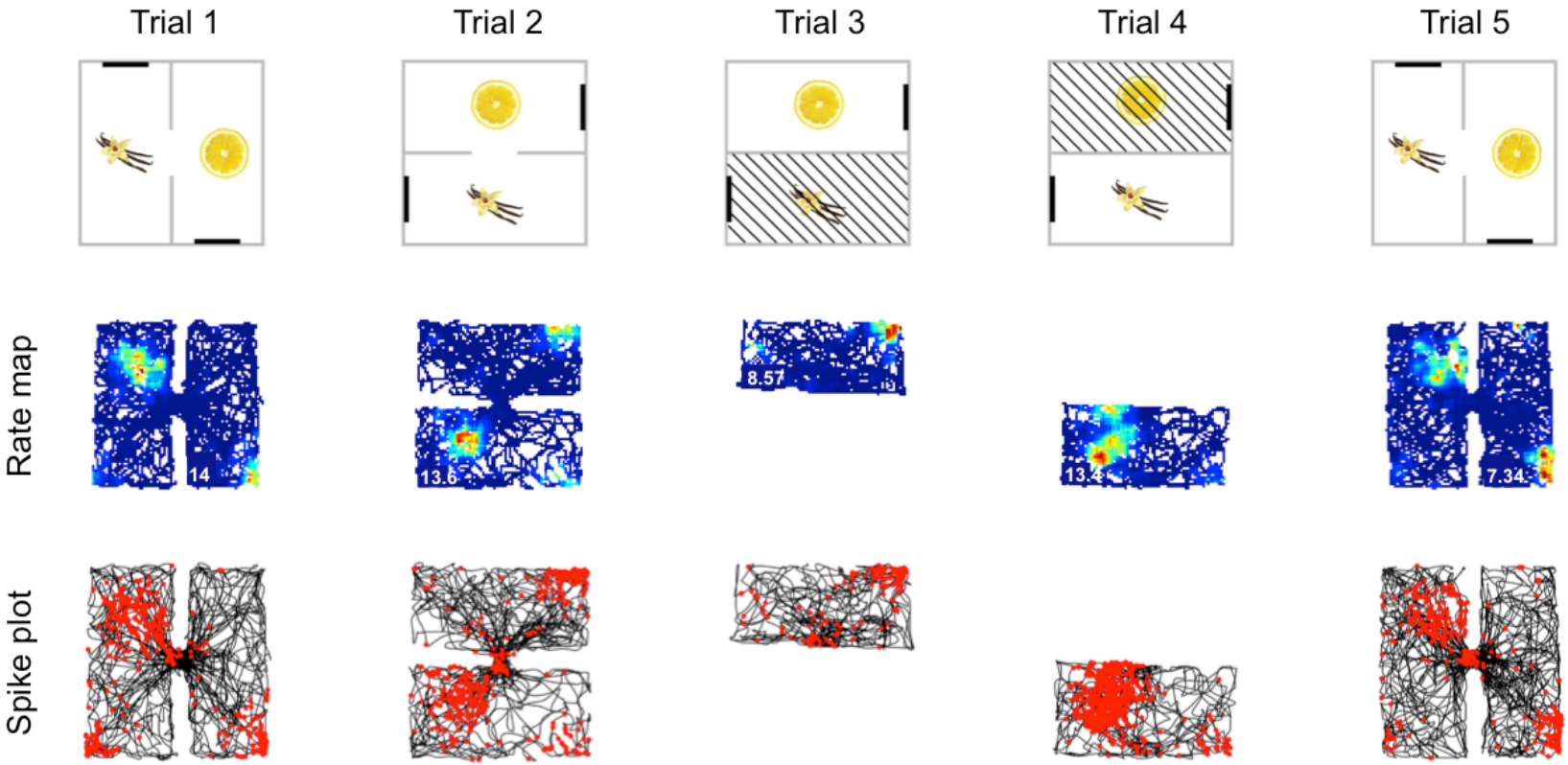


Figure 6.2: A representative cell recorded in the two-compartment apparatus that displays remapped place fields. The cell has clearly remapped place fields between the two compartments of the apparatus. These are consistent with rotation of the apparatus, and persist in the closed-door conditions of trials 3 and 4. Peak firing rates (Hz) for each trial are shown on the rate map in white.

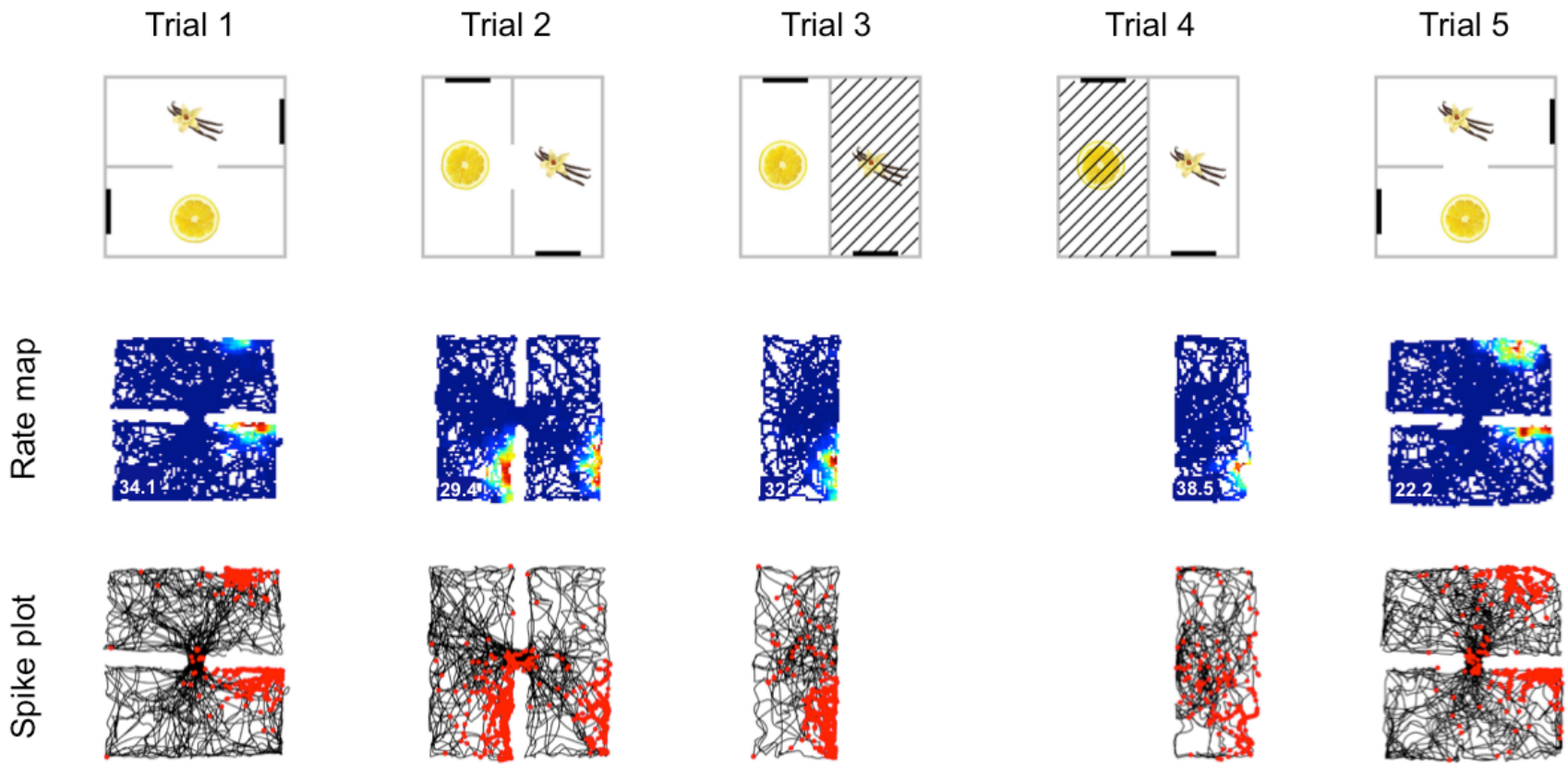


Figure 6.3: A representative cell recorded in the two-compartment apparatus that displays duplicated place fields. The cell displays duplicated place fields over the two compartments of the apparatus. These are consistent with rotation of the apparatus, and persist in the closed-door conditions of trials 3 and 4. Peak firing rates (Hz) for each trial are shown on the rate map in white.

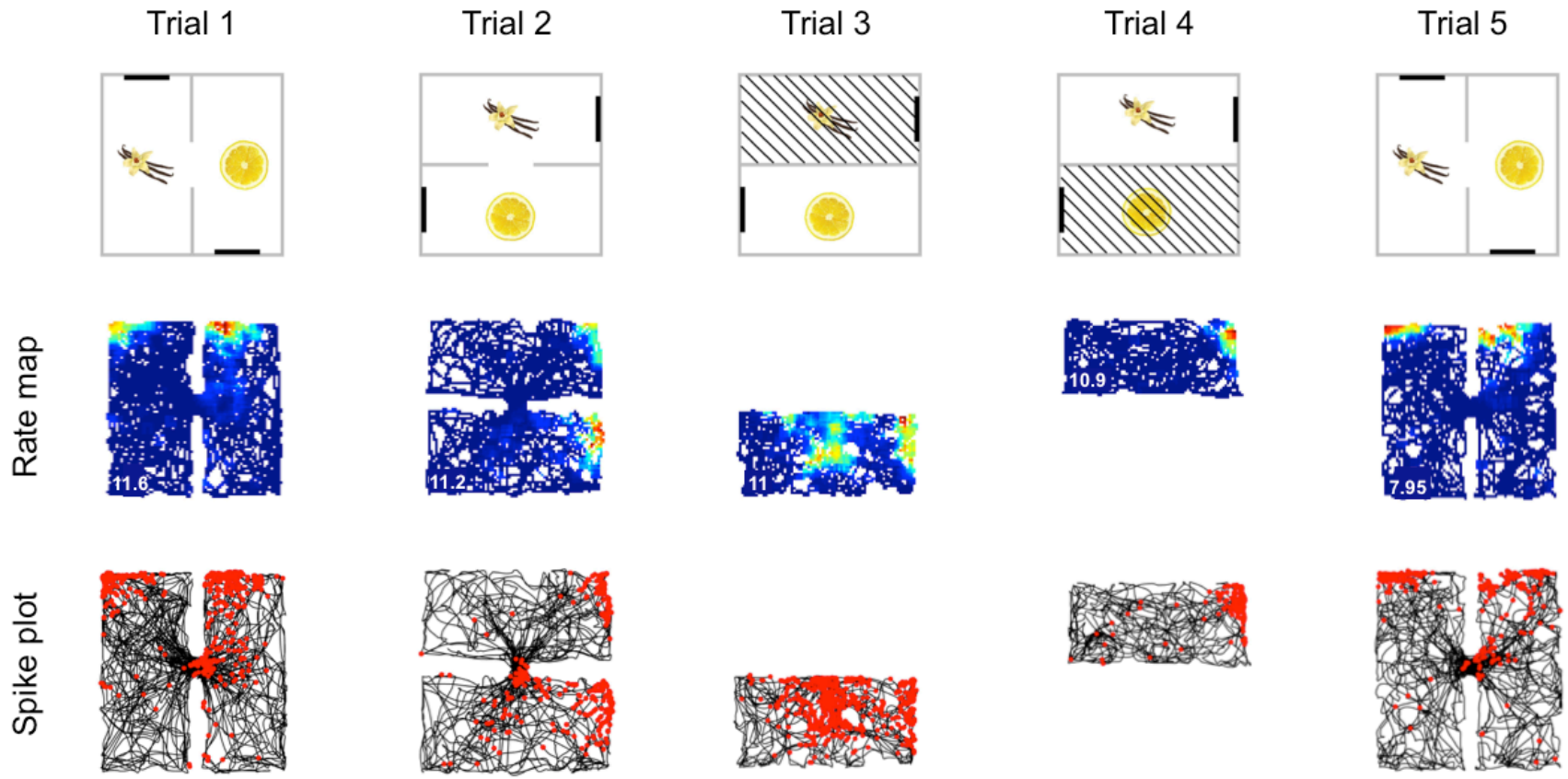


Figure 6.4: A representative cell recorded in the two-compartment apparatus that displays duplicated place fields, and local remapping in the closed-door condition. The cell displays duplicated place fields over the two compartments of the apparatus. These are consistent with rotation of the apparatus, and persist in the closed-door conditions of trials 3 and 4. Trial 3 also shows local remapping (specifically spontaneous field generation) where the barrier was introduced to separate the compartments. Peak firing rates (Hz) for each trial are shown on the rate map in white.

6.2.3 Between compartment correlation analysis

The between compartment correlation analysis confirmed the above observations of heterogeneous place field activity (see Table 2). The two compartments were correlated against one another, at 0 and 180 degrees. As expected, remapped cells returned a low correlation coefficient (*r)* at 0 degrees, while cells with duplicated fields returned a high *r* at 0 degrees (Figure 6.5, Appendix 4). The histogram in Appendix 4A shows a broad range of correlation values for remapped cells, with a peak around 0 degrees, while the duplicated field cells have a peak shifted to around 0.5 – 0.6. Results for correlation at 180 degrees were as expected, with both remapped and duplicated field cells displaying peaks at low *r* values (Appendix 4B).

One would expect that any instance of 'flip' place field activity would result in a high *r* at 180 degrees but low *r* at 0 degrees. There were some instances where the raw correlation results showed this pattern (Appendix 4B), but analysis of the map by eye discounted this as a false positive correlation; these maps did not display 'flip' fields, but instead had clear fields at the central door which were then highly correlated at 180 degrees regardless of compartment activity (Figure 6.6).

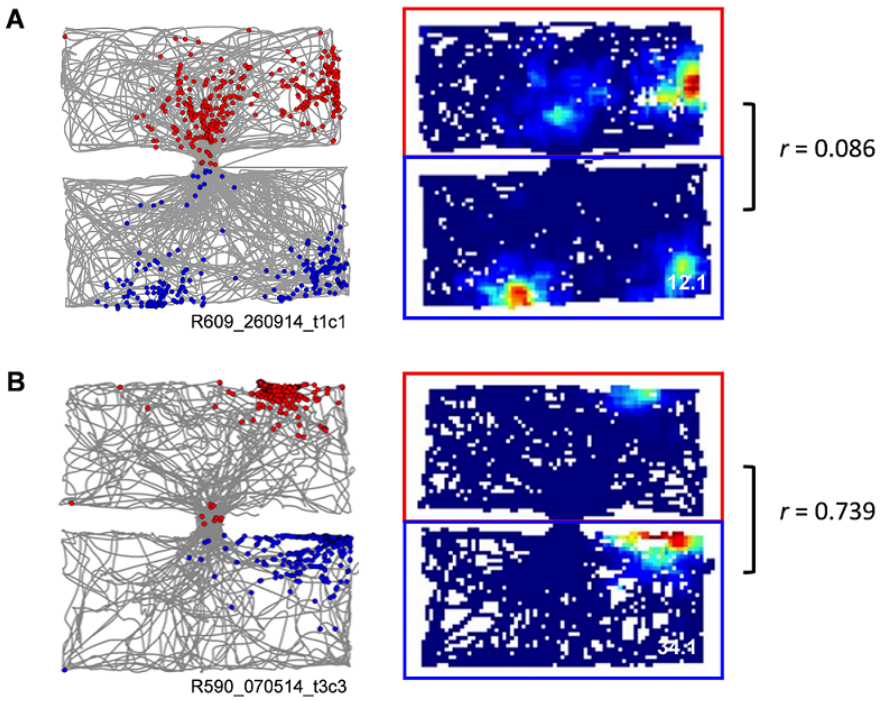


Figure 6.5: Between compartment spatial correlation analysis on place cells, at 0 degrees. The two compartments were separated along the axis with the door, such that each compartment remained intact. The spikes of one compartment are coded in red, with the corresponding part of the rate map enclosed in the same colour box; and vice versa for the blue spikes/box. The peak firing rate of the cell is stated in white at the bottom right corner of the rate map. (A) The two compartments were directly correlated at 0 degrees: this cell displayed clearly remapped firing activity and resulted in a low correlation coefficient (*r*). (B) When directly correlated at 0 degrees, cells with duplicated fields resulted in a high *r*.

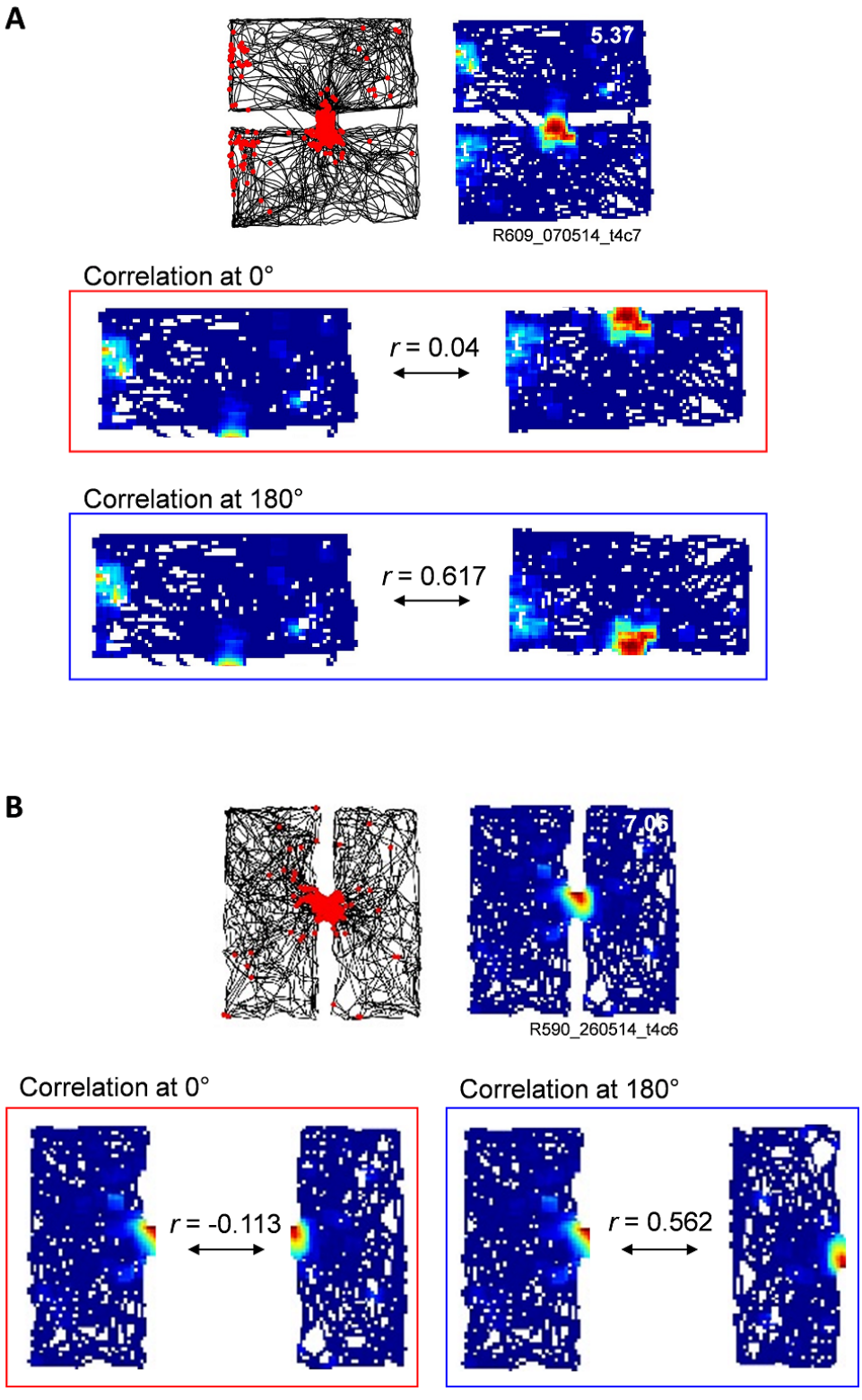


Figure 6.6: Between compartment correlation at 0 and 180 degrees. To look for the existence of place cells with 'flipped' (or mirrored) fields between compartments, the rate maps for each cell were halved and correlated at both 0 and 180 degrees. It was expected that 'flip' field cells would have a high correlation coefficient (*r*) at 180 but a low *r* at 0 degrees. By eye, no cells were found to have flipped fields but the between compartment correlation returned some cells with the expected pattern. However, these cells were similar to the examples above: that they all exhibited central 'door' fields and thus regardless of activity outside the door, they displayed a high correlation value at 180 degrees and were false positive for 'flipped' field activity.

6.2.4 Place fields in the two-compartment box are evenly distributed

Plotting the weighted centre of isolated place fields (this process was detailed in 4.4.3.8) showed that place fields are fairly equally distributed over the entire environment, with no clear preference to one area (e.g. closer to walls) (Figure 6.7). There is also no distinct bias in the number of place fields between the two contexts.

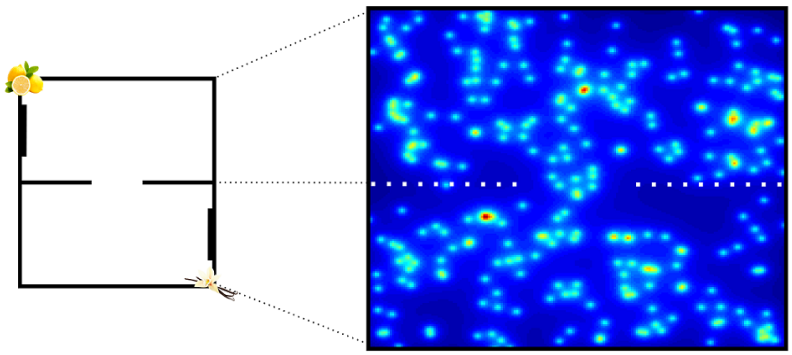


Figure 6.7: Locations of place field centres recorded in the two-compartment box. The locations of place field centres from all recorded place cells were extracted using custom MATLAB scripts based on the criteria in 4.4.3.8. Place field locations appear to be well distributed over the whole environment, with no clear clustering of activity in a given region.

6.2.5 Place cells can maintain their representations in single context conditions

In order to further test the theory that place cells can use odour context information to drive their spatial representations, the animals were exposed to each compartment separately in trials 3 and 4, and were unable to move freely between the two. If the place cells were able to use odour context information to separate the two compartments, they may also be able to use the remaining odour and distal cue in the single compartment condition to maintain a stable spatial representation.

Observations of cell behaviour in the 'closed trial' conditions showed that this was indeed the case: place cells were able to retain their spatial representation of the lemon and vanilla compartments when only allowed to move in a single compartment (Figure 6.2, 6.3, 6.4). Correlations between baseline and closed-door trials (performed as in 4.4.3.7) confirmed that the level of stability between trial 1 maps and trial 3/trial 4 maps was above the remapping threshold of 0.3 (stable cells: average t1/t3 = 0.471; average t1/t4 = 0.511). There was no bias of odour in the ability to retain the same spatial representation in the closed-door trials (no difference between t1/t3 and t1/t4 correlations, $p = 0.632$; one way ANOVA with post-hoc Tukey HSD). A lack of stability of the cell across the whole experimental day had little effect on spatial representation

in closed trials, with results still 0.3 or over (unstable cells: average t1/t3 = 0.420; average t1/t4 = 0.300).

Interestingly, while the closed trials are classed as stable compared to trial 1, the correlation values obtained are significantly lower than those from correlated baseline trials [$F(3,427) = 26.1$, $p < 0.001$; $p < 0.001$ for trial 1/2 vs trials 1/3 and 1/4; one way ANOVA with Tukey HSD] (Figure 6.8A). Correlations to trial 5 were not performed here due to the previous role of the T1/T5 correlation in classifying cells into 'stable' and 'unstable' groups (see section 4.4.3.6). This difference is most likely down to an observed incidence of local remapping (specifically, spontaneous field generation) in the single compartment trials, similarly to the results of Alvernhe et al. (2011, 2008) upon the introduction of barriers. However, this effect was not significant for the unstable populations of cells, likely due to the small population size and higher variance in correlation values (one way ANOVA with Tukey HSD; [$F(2,44) = 2.5$, $p = 0.094$]; Figure 6.8B).

Histograms of the results presented in Figure 6.8 can be found in Appendix 6.

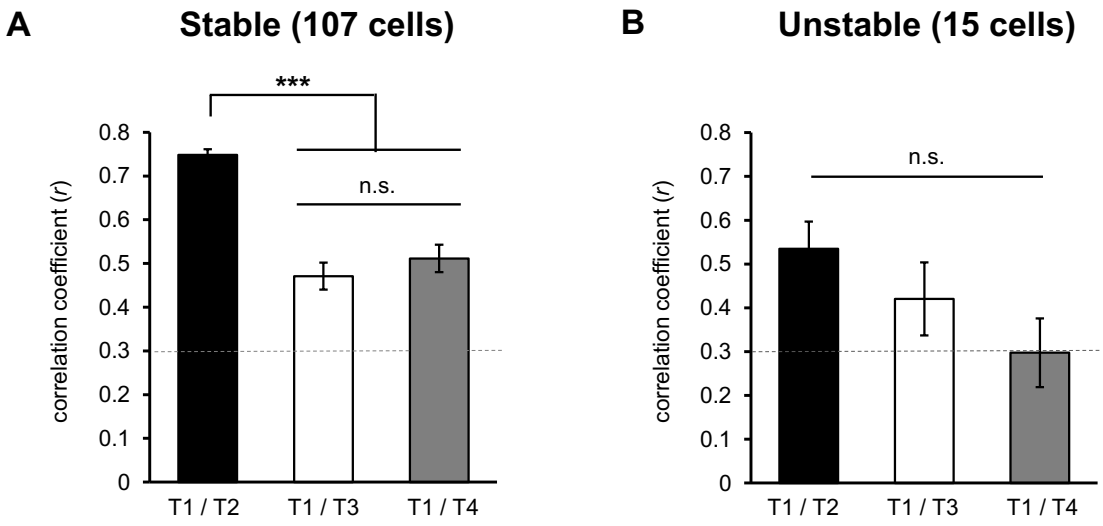


Figure 6.8: Place cells can retain stable representations of space when only exposed to a single compartment. Dashed line at r=0.3 represents the remapping threshold. (A) The closed-door trials of stable cells retain the same spatial representation as trial 1, with no bias from odour. However, the correlation values for closed-door trials are significantly lower than when trial 1 is correlated with the rotated baseline of trial 2. This is likely to be due to local remapping (generation of fields) following the introduction of barriers as Alvernhe et al., 2008, 2011. (B) Unstable cells do not follow the same pattern, with no significant difference between any of the correlation pairs, however the closed trial correlations remain at or above 0.3 suggesting stability of the representation.

6.2.6 Histology

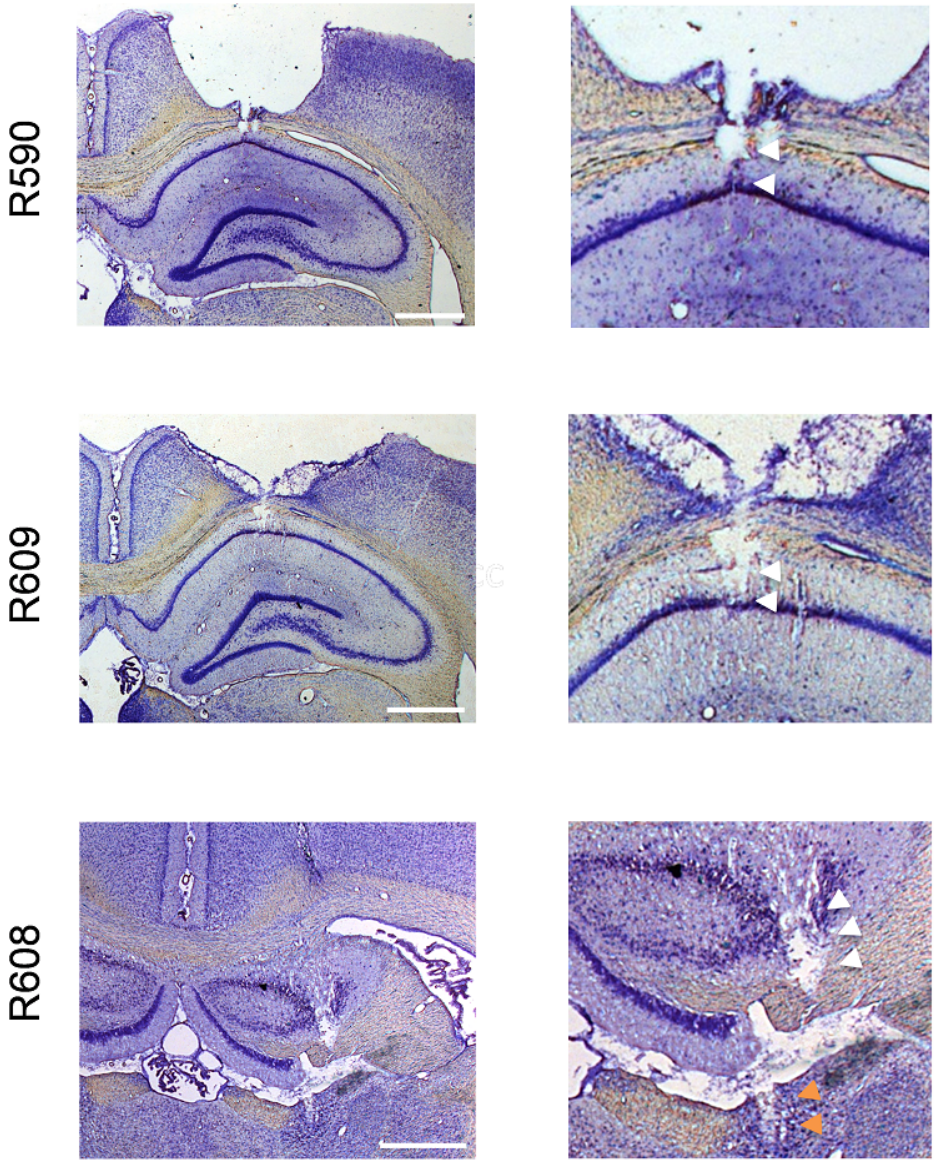


Figure 6.9: Histology for three rats. Left) Stained coronal slices for each rat at 2.5x magnification, showing the general area of interest (R590, R609 show the hippocampus proper, while R608 shows both the hippocampus and anterior thalamus). Scale bar in each image represents 1mm. Right) Images at ~10x magnification for clearer view of tracks. White arrows indicate electrode tracks through the hippocampus; orange arrows indicate tracks in the ADN.

6.3 Discussion

In this experiment, rats freely explored two connected, identical, but visually rotated compartments that could be distinguished by a combination of odour (lemon/vanilla) and directional information. We observed heterogeneous responses in place cell activity between the two compartments: there was a population of cells presenting with differential firing in each compartment (remapping); and another with identical firing in each compartment; but no evidence of 'flip' place cells with rotated firing that was expected from the aforementioned evidence of RSC head direction cell response under these conditions. This suggests that place cells are able to use context information to resolve the directional ambiguity, but also indicates that the only head direction information entering the hippocampus is from a single global signal. The following discussion will put these results in perspective with the current literature.

6.3.1 Place fields are sensitive to odour information

Previous studies have shown that place cells (and grid cells) exhibit repeated fields across compartments having similar geometry (Skaggs and McNaughton, 1998; Fuhs et al., 2005; Derdikman et al., 2009; Spiers et al., 2015), suggesting that place cells are influenced more strongly by local cues such as boundaries (O'Keefe and Burgess, 1996), than by path integration information as the animal moves between compartments.

In the Fuhs et al. (2005) study, the authors observed a higher incidence of place field remapping than place field repetition in a similar two-compartment environment with a 180° offset. However, this higher incidence of remapping could have been a product of their methodology. Firstly, the animals were familiarised with both compartments in a parallel configuration (identical orientation), which, as expected, resulted primarily in place field repetition across compartments. In this condition, as in the following 180° offset condition, the animals were always placed in the same compartment (Box A) and confined to it before gaining entry to the other compartment (Box B), never being allowed to move freely between the two compartments. As they consistently placed the animal in Box A at the beginning of each trial, and the box A representation was stable while box B displayed remapping in the absence of any defining cues, it would suggest that the place cells were using this repetitive initial transfer into the environment to fuel their recognition of boxes A and B as separate compartments (Sharp et al., 1990).

The present study confirms and builds upon these results by showing that the animals can innately disambiguate between both compartments when distinct context information (odour) is available. The majority of place cells (86.8% of total recorded)

display remapping between compartments. This remapping presented as either two distinct place field representations in each compartment (Figure 6.2) or place field activity only in one compartment with the cell silent in the other. Second, conversely to the earlier study, place cells with remapped fields were seen from the first exposure of the animal to our two-compartment apparatus. One possible explanation for the absence of the delay in onset of remapped fields seen by Fuhs et al. (2005) is that our animals were allowed to move freely between the compartments as frequently as they wished many times, and had the reinforcing component of two separate odour contexts.

Similarly to previous studies, we also observed place field repetition in a small number of place cells (13.1% of total recorded). Place field repetition despite the presence of orienting odour cues is consistent with the proposal of Anderson & Jeffery (2003) that the contextual information is received by hippocampal CA1 place cells in separate parts rather than as a single pre-assembled 'context signal' for each environment. They found heterogeneous remapping in response to compound context (*i.e.* some cells responded only to odour in a context combination or only to colour), suggesting that neighbouring neurons do not all receive the same contextual information and that there is a level of detection of the individual signals within the compound context. In the case of our population of place cells with repetitive fields, it is likely that these cells were not influenced by the odour and instead received stronger inputs from boundary vector cells (Hartley et al., 2000; Lever et al., 2002). Given that boundary vector cells are sensitive to allocentric direction, a change to the rat's internal orientation would result in place cell remapping, but as the majority of head direction cells provide a global and consistent head direction over both compartments (Jacob et al., 2016); see also Figure 3.6) then these cells will likely present with repeated fields.

There is also a possibility that these place cells with duplicated fields are undergoing another type of remapping known as 'rate remapping' (Muller & Kubie, 1987; Bostock et al., 1991; see also section 3.2.1) where the difference between compartments would be noticeable in a consistent disparity over trials between maximum firing rates. Due to the small sample size of these cells recorded (16 out of 122 cells), and having only three trials for comparison, the analysis was not conducted.

6.3.2 Hippocampus can maintain spatial representations based on odour context information

The results of the 'closed-door' trials (trials 3 and 4) indicate that the hippocampus can process odour-context information to maintain a representation of each compartment

separately *i.e.* represent the 'lemon compartment' activity without access to the second compartment, as the closed-door representation stably correlated to baseline place field activity. Thus the place system can use odour to establish a directional orientation in this environment. Since the odour information itself is non-directional, the ability to do maintain these spatial representations must have come from learning in the preceding open-door trials.

A notable observation from these closed-door trials is that local remapping often occurs following the introduction of the barrier to close the door, similarly as in experiments conducted by Alvernhe et al., (2008; 2011). These studies noted that where there were alterations to the environment, either the introduction of barriers or removal of sections of the walls, there was also a change to the place cell firing fields close to these manipulations. The predominant observation from the aforementioned studies was that fields close to these alterations enlarged in size, often encompassing the altered area, while fields further away were largely unaffected. Similarly, in the current study, place fields around the 'ex-door' area often enlarged and the fields far from the door were unchanged (Figure 6.6). Local remapping also often presented as new fields appearing. However, in contrast to Alvernhe et al. (2008; 2011), we did not observe that the local remapping changes to place fields disappeared post-manipulation.

6.3.3 Summary

This study has confirmed that hippocampal place cells, and globally-encoding HD cells, can resolve the directional ambiguity of the two-compartment apparatus, and that, in this apparatus, this ability involves processing of contextual information. We have also confirmed that the hippocampus responds heterogeneously to this contextual information. Given these findings, it is plausible that the globally-encoding head direction cells in the retrosplenial cortex (alongside the rest of head direction network including the ADN and PoS) may predominantly reflect the 'sense of direction' passed through the spatial network, with information from the bipolar head direction cell population being filtered out by the time the directional signal reaches the hippocampus. This hypothesis will be revisited in the final discussion (Chapter 8), after the presentation of the following experiment which reinforces the role of the HD system in the task.

Chapter 7 - Experiment 3

Does resolution of directional ambiguity in the location-odour task depend on the head direction system?

The two-compartment context box provides an environment with directional ambiguity that can be resolved either by the use of head direction cues or odour context information (or a combination of both). The novel location-odour task developed in **Experiment 1** showed that animals could use odour information to guide their behaviour and resolve the ambiguity presented by the visual symmetry of the task, but it gave no insights into how the animals achieved this.

The results of **Experiment 2**, in which place cells displayed partial remapping and no incidence of rotated fields defined by local cues, suggested that the hippocampal place system was able to use the global head direction signal (Jacob et al., 2016) together with the olfactory cues to orient its spatial representation of the two-compartment box. This lent more support to the hypothesis that the ability of the animals to resolve the visual symmetry in the location-odour task involved the HD system.

To test this hypothesis, we opted to temporarily inactivate the HD system through microinfusion of muscimol to the anterior thalamus (AT). Pharmacological intervention is preferable over a permanent excitotoxic lesion because a) it allows animals to act as their own controls due to its reversible nature, b) it causes less effect of compensation, as the inactivation is short-lived, and c) it affords a level of temporal precision for the inactivation within the experiment (Majchrzak and Di Scala, 2000; Bonnevie et al., 2013) . Similar microinfusions of muscimol have been used successfully to inactivate the AT, under behavioural paradigms testing both spatial navigation and context memory (Law and Smith, 2012; Stackman et al., 2012). Muscimol, a $GABA_A$ agonist, was used in the present study to disrupt the classical directional signal by inhibiting head direction activity in the AT; this structure was chosen because it acts as a hub for ascending and descending streams of the head direction signal and thus would cause widespread network disruption (Taube, 2007).

The design of the present experiment was identical to that of **Experiment 1D**, except for the addition of the drug infusion prior to all of the trials (habituation and probe) on the final day of testing (Figure 7.1). The drug was given only on the day of the probe trial in order to remove the possibility that the animals would not react to the probe test because they were not sufficiently habituated. The 30-minute rest period post-infusion allows for the drug to become effective (Bonnevie et al., 2013), so the seven

habituation trials on the final day of testing serve to confirm that the dose of muscimol does not have an effect on animal behaviour or the amount of exploration.

We hypothesise that the ability of the animal to resolve the directional ambiguity presented by the test trial of the location-odour task is mediated by the head direction system. If the muscimol-infused animals (*i.e.* with a disruption to the HD system) fail to recognise the spatial displacement at test (observed by re-exploration of the displaced object), but are still able to recognise novelty per se, this would be a strong indication for acceptance of this hypothesis.

7.1 Methods

7.1.1 Subjects

Eight naïve adult male Lister Hooded rats were used in this experiment, at least 8 weeks of age and 350 grams in weight prior to surgery.

7.1.2 Surgery

Refer to section 4.3.3 for detailed methods of '*Anterior thalamus cannulation'* and other common surgical procedures.

7.1.3 Experimental apparatus

The two-compartment context apparatus is described in 4.4.1 . Circular opaque curtains surrounded the apparatus so that no distal cues (external to the recording environment) were available to aid animal navigation.

7.1.4 Behavioural testing

Figure 7.1: Timeline of the inactivation experiment (Experiment 3). Animals were given four days of habituation to the two-compartment context box with both odours and objects, at a rate of eight trials per day. Following this, the animals were given a microinfusion of drug (or saline in sham animals) before the final testing day; this 'drug day' consisted of another seven habituation trials and a final probe trial as the eighth. Drug animals were also given a second probe trial (drug day, trial 9) where the spatial displacement was reversed but the object was replaced with a novel one.

The behavioural paradigm was the same as outlined in Experiment 4 (Figure 6), with an additional trial on the test day for drug infused animals (Day 5, trial 9). This additional trial was designed to ascertain whether the animals retained normal response to novelty – the spatial and visual ambiguity was removed, and the previously displaced object was replaced with a completely novel one. Thus in the two probe trials, we tested both spatial (a familiar object in a novel place) and object recognition (a novel object replacing a familiar one) (Eacott and Norman, 2004; Langston and Wood, 2010); accurate recognition of a change was indicated by preferred exploration of the object that occupied the new location or the novel object. Animals acted as their own controls and were part of a randomised crossover paradigm; treatment assignments were randomised and reversed for the second infusion so that each animal received both treatments. A 3-week period was left between each instance of

testing (sham or drug), and different objects were used for each instance. The second set of objects was of a similar shape and size to the first, but was a different colour and had different pattern of textures.

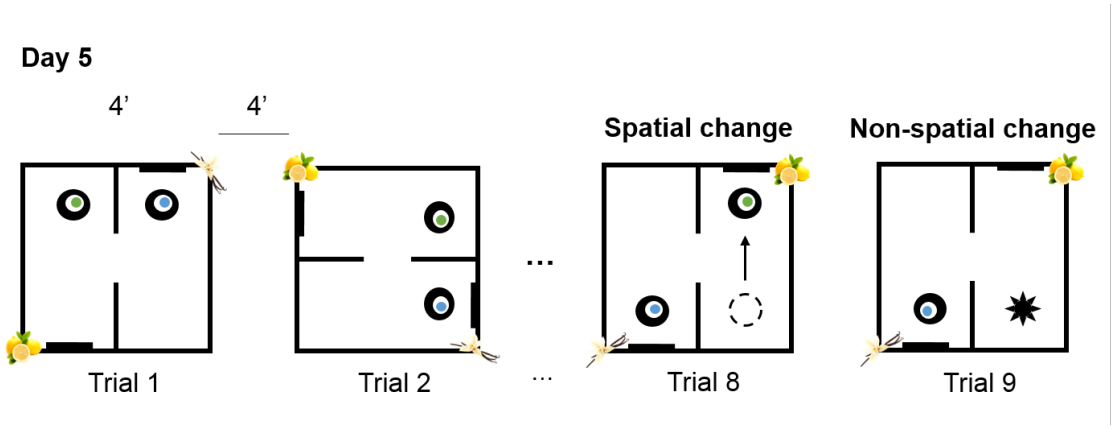


Figure 7.2: Behavioural protocol for the testing day (Day 5) in Experiment 3. The final day of testing consisted of seven habituation trials, followed by a test trial (Day 5, trial 8) where a single object was displaced to restore visual symmetry to the environment. In this test trial of spatial change, the animal must rely on complex cue integration of odour to resolve the directional ambiguity. To assess standard novelty response, a final trial was introduced for animals receiving muscimol only: in this trial (Day 5, trial 9), a non-spatial change was performed where the spatial ambiguity was removed and the previously displaced object was switched with a novel one.

7.1.5 Intracranial microinfusion

To reversibly inhibit neurons in the anterior thalamus, we implanted rats with bilateral guide cannulae above the structure (see section 4.3.3). Before the first trial on the test day, each rat was gently restrained, the dummy cannula removed and a 33-ga bilateral infusion cannula (Plastics One, Bilaney Consultants, UK) inserted through the guide cannula. Each infusion cannula protruded 1mm beyond the tip of the implanted guide cannula to reach the structure. Each injector was connected by length of polypropylene tubing to a 10-µl syringe (1801, Hamilton Company) mounted in a double syringe infusion pump (Pump 11 Elite, Harvard Apparatus).

Temporary inactivation was induced with the $GABA_A$ agonist muscimol. Thirty minutes prior to behavioural testing, muscimol (Sigma Aldrich, UK) dissolved in 1% PBS (pH 7.4; concentration 0.5 µg/µl) was infused bilaterally into the anterior thalamus; bilateral infusions were performed simultaneously at an infusion rate of 0.08 µl/min and a total volume of 0.24µl for each infusion. The internal cannula was retracted 2 minutes after the infusion.

For sham animals, the muscimol was replaced with 1% PBS vehicle for the infusion. All other procedures remained the same as above.

7.1.6 Microinfusion of fluorophore-conjugated muscimol

To determine the degree of tissue distribution of microinfused muscimol at test, each animal received a microinfusion of fluorophore-conjugated muscimol (0.5ug/ul, BODIPY TMR-X, Invitrogen) prepared in PBS at the end of all behavioural testing (for method, see section 7.1.5). Post-infusion, the animal was returned to the home cage for 1 hour to simulate the time delay to the probe trial of the test day. Animals then underwent transcardial perfusion with NaCl followed by 10% formalin, and the brains were retrieved for histological analysis (detailed in section 4.5.1).

7.1.7 Data analysis

Data were analysed as in Experiment 1 (Chapter 5); for details see section 4.4.2.2.

7.2 Results

Baseline exploration within and between days is consistent between groups

For each group, sham and drug, the exploration time of each object was assessed during the habituation phase to see whether there was any bias caused by a specific odour. Neither group showed a significant preference for one odour over the other (Figure 7.3A and B; repeated measures ANOVA: sham, [$F(1,14) = 1.499$, $p = 0.241$]; muscimol, [$F(1,14) = .082$, $p = .779$]).

As exploration time did not vary significantly in response to odour, the exploration times of the vanilla and lemon objects were pooled within each trial to obtain a total exploration time measure. The total exploration time was averaged in blocks of eight trials, reflecting the number of trials in each experimental day; the eight trials of each of Days 1 to 4, plus trials 1- 7 of the 'drug day' (Day 5) were classed as the 'habituation phase'. During the habituation phase, exploration values were no different between the groups thus reflecting an similar learning and behaviour pattern (Figure 7.3C; repeated measures ANOVA, [$F(1,14) = 3.508$, $p = .082$]).

Animal exploration across days in the habituation phase was significantly different in both groups (repeated measures ANOVA: sham, [$F(9,63) = 8.17$, $p < .001$]; muscimol, [$F(9,63) = 2.12$, $p = .04$]). Both groups also showed negative trends in object exploration across the full experiment, between days, when analysed using the directional Jonckheere test (Jonckheere and Bower, 1967) (sham, $Z = -3.56$, p (2-tailed) < 0.001; muscimol, $Z = -2.72$, p (2-tailed) < 0.01).

However, within-day trend tests noted variation in Z scores for each day in both groups (see Table 3). Despite consistently negative Z scores, indicating a trend of decreasing exploration values over the habituation trials in a day, there is inconsistent variation in the significance levels for these trends in both groups. This suggests that while the animals did display learning over the full five-day experimental paradigm, that this learning was not linear for either the sham or drug group.

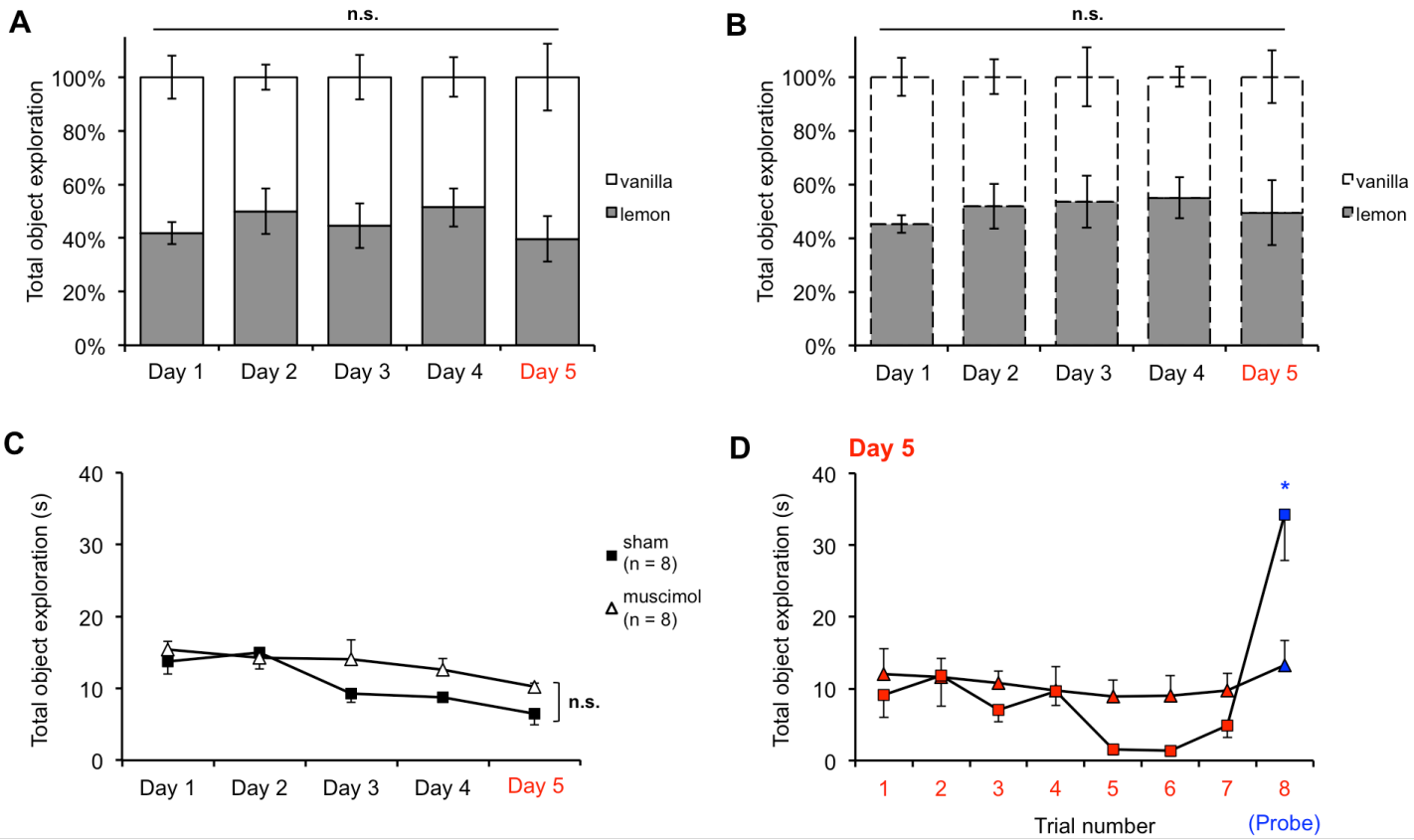


Figure 7.3: Baseline exploration, regardless of odour context, is the consistent between groups but the probe trial elicits a differential response. Red colour (text or marker) indicates any trials corresponding to the fifth experimental day (Day 5), also termed 'drug day'. Blue colour (text or marker) indicates the probe trial. (A) The total exploration time for sham animals over all five days, represented by the proportion of lemon and vanilla object exploration (mean±SEM), does not significantly differ between olfactory contexts [$p > .05$] (B) Similarly, the total exploration time for muscimol animals over the five days also does not significantly differ between olfactory contexts [$p > .05$] (C) Pooled total object exploration over the habituation phase (Days 1-4, and trials 1-7 of Day 5) does not differ between groups [$p > .05$] (D) Pooled total object exploration demonstrates a large difference between object exploration of the sham and drug groups in the probe trial (drug day, Day 5, trial 8) ($p < .05$).

	Sham (n=8)			Muscimol (n=8)		
	Z score	*p* (2-tailed)	Significance	*Z* score	*p* (2-tailed)	Significance
Day 1	-3.56	3.58×10^{-5}	***	-3.25	0.001	***
Day 2	-3.70	0.0002	***	-1.51	0.130	n.s.
Day 3	-1.86	0.064	n.s.	-2.83	0.005	**
Day 4	-1.68	0.093	n.s.	-2.20	0.028	*
Day 5	-3.01	0.003	**	-1.35	0.177	n.s.

Table 3: Distribution of *Z* scores and significance levels for within-day trend tests. Within-day Jonckheere trend tests revealed purely negative trends (indicated by negative *Z* scores) but varying levels of significance over days for both groups. This indicates learning occurred in both groups but that this trend was not linear over experimental days.

Probe trial elicited re-exploration in sham but not drug animals

In the probe trial on the 'drug day' (Day 5, trial 8), there was a significant difference in raw exploration time between the drug and sham groups (Figure 7.3D) (average raw exploration values: sham, *M* = 34.3s, *SE* = 6.39s; muscimol, *M* = 13.3s, *SE* = 3.49s) (paired T-test (2-tailed), $t(7) = 3.00$, $p = .02$). To explore this difference in relation to exploration in the habituation sessions, we created a baseline for exploration by averaging trials 1 to 7 of the drug day. A two-way ANOVA of drug and test condition (baseline or probe trial) was then performed to compare baseline exploration to probe trial exploration for each group: this highlighted a significant effect of drug [$F(1,28) = 5.04$, $p = .033$] and test condition [$F(1,28) = 16.5$, $p < .001$], as well as a significant interaction [$F(1,28) = 10.7$, $p = .003$]. Thus, only sham animals showed a significant increase in time exploring objects.

We then tested to see whether the displaced and non-displaced objects were explored differentially by each group. Raw exploration data shows that on average 93% of the total exploration in the probe trial was of the displaced object in the sham group (sham, average exploration values: displaced, *M* = 31.9s, *SE* = 6.38s; non-displaced, *M* = 2.38s, *SE* = 0.96s), and in the drug group this value decreased to 64% (muscimol, average exploration values: displaced, *M* = 8.38s, *SE* = 2.97s; non-displaced, *M* = 4.88s, *SE* = 1.53s).

The D2 discrimination ratios for the drug day reflect the raw data and show that there is a significant difference exploration between objects over the experiment in the sham group (one-way ANOVA, [$F(7,63) = 2.796$, $p = .014$]; Appendix 3B) but not the muscimol group (one-way ANOVA, [$F(7,63) = 0.750$, $p = .631$]; Appendix 3C).

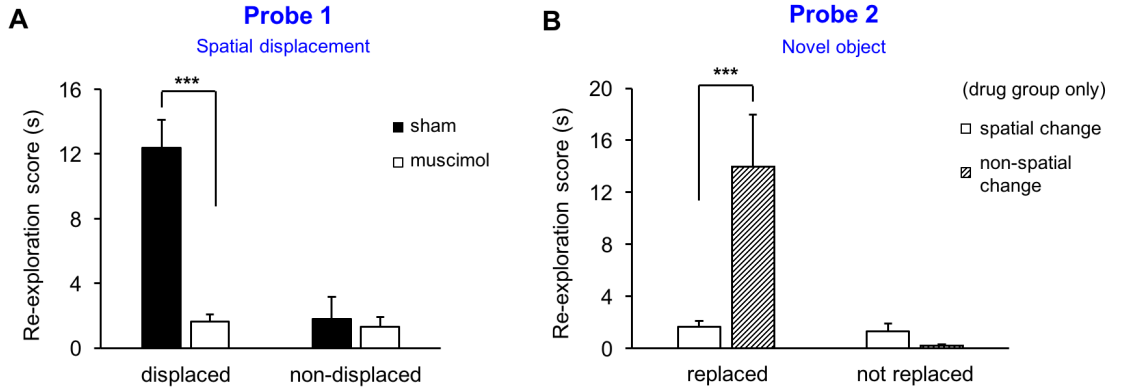


Figure 7.4: Exploration of the spatially displaced object is significantly higher in sham animals, while animals with disruption to the HD system can recognise novelty per se but not spatial displacement. (A) Re-exploration values for the probe trial between sham and drug groups: spatial displacement induced re-exploration of the displaced object in sham animals, but drug animals did not react to the change. (B) To assess whether the drug animals retained a novelty response, an additional probe trial was conducted where the familiar object was replaced, with no spatial displacement, with an entirely novel one: muscimol-treated animals displayed re-exploration of the novel object, so they can respond to novelty per se but not spatial displacement. Sham animals were not given this test so their data is not presented here.

To confirm that the discrimination results were not subject to noise from low exploration values, the re-exploration score was also calculated for each object in relation to the baseline exploration of that object in the habituation trials (see section 4.4.2.2 for detailed explanation of this calculation). Consistently with the discrimination ratio, re-exploration scores in the probe trial, drug day trial 8, demonst rate a significant difference between the performance of sham and muscimol-treated animals (Figure 7.4A) (average re-exploration scores: sham - displaced, M = 12.4s, SE = 1.72s, non-displaced, M = 1.79s, SE = 1.36s; muscimol - displaced, M = 1.63s, SE = 0.46s, non-displaced, M = 1.31s, SE = 0.60s) (two-way ANOVA (drug and displacement), significant effect of drug [$F(1,28) = 23.5$, $p < .001$], displacement [$F(1,28) = 22.2$, $p < .001$], and a significant interaction [$F(1,28) = 19.6$, $p < .001$]). This suggests that sham animals re-explore the displaced object following spatial displacement, but the animals with inactivated anterior thalamic nuclei do not explore above a baseline level.

HD system disruption impairs recognition of a novel location under directional ambiguity not recognition of novelty per se

To confirm that the lack of re-exploration was not due to a non-specific inability to recognise novelty, the drug group were subsequently exposed to a second probe trial where a familiar object was replaced with a novel one without spatial displacement. Re-exploration scores for this second probe trial (Day 5, trial 9) show a significant difference when the animals' performance was compared to their performance in the

initial probe trial (Day 5, trial 8) (Figure 7.4B) (raw re-exploration scores: spatial change, *M* = 1.63s, *SE* = 0.46s; non-spatial change, *M* = 13.9s, *SE* = 4.03s) (two-way ANOVA, significant effect of type of change (spatial or non-spatial, [$F(1,26) = 8.55$, $p = .007$]), displacement [$F(1,26) = 13.5$, $p = .001$], and a significant interaction [$F(1,26) = 12.3$, $p = .002$]). this means that muscimol-treated animals, with a disruption to the HD system, can recognise object novelty but cannot reconcile the spatial ambiguity created by spatial displacement.

To summarise, exploration during the habituation phase was not significantly different between the sham and drug groups, and both groups demonstrated a negative trend in exploration over the five experimental days. This suggests that they became equally familiar with the objects and contexts in the environment. Under the effect of the drug, animals showed no difference in performance to the sham during the habituation trials of the testing session; this indicates that the drug does not cause bias to increased/decreased exploration or preference of one object over another. However, at test, sham animals re-explored the spatially displaced object while animals with disrupted HD systems did not, and this effect was not due to lack of novelty response. These findings suggest that the HD system is involved in, and maybe necessary for, the ability of the animals to use these complex location-odour representations to resolve directional ambiguity using context.

7.2.1 Histology

Fluorescent muscimol infusions were inconclusive; therefore spread of the drug in these animals could not be determined. However, from previous studies using similar dose and volume of muscimol, an estimation of spread can be made that agrees with coverage over the anterior thalamus (including anteroventral and anterodorsal thalamus) (Law and Smith, 2012). Infusion sites were confirmed through Nissl staining, and are shown in Figure 7.5.

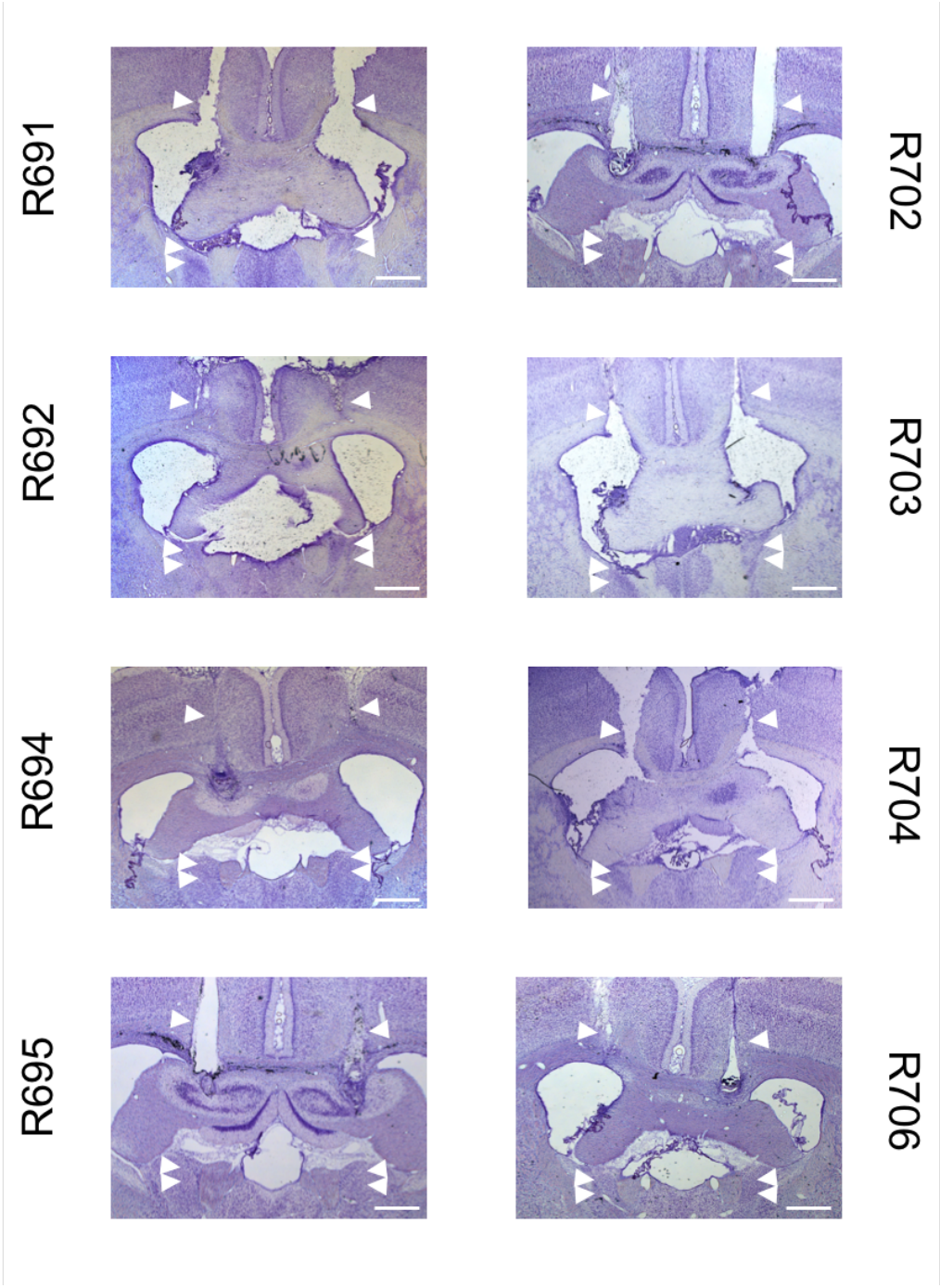


Figure 7.5: Histological verification of bilateral infusion cannula placement into the anterior thalamus. Photomicrographs of nissl stained brain slices for each animal in Experiment 3, showing tracks for the chronically implanted cannula, implanted to reach 1mm above the anterior thalamus. White triangles show the position of the cannula track and the path of 1mm long injector cannula. Scale bar = 1mm.

7.3 Discussion

As shown in Experiment 1 (Chapter 5) of this thesis, animals are able to use olfactory context information to guide their behaviour. While the spontaneous recognition paradigm is designed to exploit the rat's tendency to explore novelty, this version of the task also requires the animal to resolve a directional ambiguity using only context cues. Here, the spatial displacement could only be recognised had the animal correctly encoded a complex location-odour conjunction. Findings from the sham group tested in this experiment, and experiment 1, add to limited evidence that animals can use purely odour context to drive spatial recognition (Anderson et al., 2006; Save et al., 2000; Wallace et al., 2002), shown by preferential re-exploration of the displaced object at test. The present experiment added to this finding by showing that a manipulation causing disruption to the anterior thalamus (AT) resulted in abolition of this preferential re-exploration. Thus, this suggests that the anterior thalamus, and likely the head direction system as a whole, is necessary for encoding these complex location-odour configurations and resolving directional ambiguity.

Of the two ways it was possible that animals were solving this task, there were two hypotheses: animals could use a non-spatial method to form a compound representation of landmark, object and odour (O'Reilly and Rudy, 2000), meaning that disruption of the HD system would have no effect on task performance; or, they may be using the head direction cues and odour cues together to generate complex context representations(Jacob et al., 2016), such that disruption of the head direction system would cause deficits in task performance. The results of Chapter 6 show that place cells can use odour to support a directional representation, so in principle the animal has the information needed to solve the task using a true spatial strategy. The results of this chapter add great support to this possibility, showing that resolution of directional ambiguity in the location-odour task is in some way reliant on an intact AT (and by extension, an intact head direction system).

Though primarily known for its crucial role in the head direction system, AT has also been shown to be important for context memory in rats (Law and Smith, 2012). In a context-based odour discrimination task, the AT was shown to be critical to contextual processing such that any performance advantage afforded by learning in two different contexts was abolished under lesions of the AT. The very similar abolishment of contextual advantage was obtained when the same task was run under inactivation of the hippocampus (Butterly et al., 2012). Additionally to this, current literature indicates that animals with impaired HD systems (and by extension disruption of the AT, or specifically the ADN) often cannot perform at above-chance levels in spatial tasks

(Aggleton et al., 1996; Stackman et al., 2002; Wilton et al., 2001), and that the stability and coherence of hippocampal place fields (leading to stable representations of space) are dependent on directional information (Calton et al., 2003). Taken together, this suggests that the head direction circuit and the interconnected network known for spatial processing (including the hippocampus, RSC, PoS and EC) quite likely may also be part of a functional circuit involved in contextual memory.

Disruption to the interconnected network of the AT

Intrathalamic microinfusion of muscimol, a non-selective $GABA_A$ agonist, would have indiscriminately inactivated both directional (~60% of total number; Taube, 1995) and non-directional neurons in the anterior thalamus. It is therefore important to note that response to disruption of the anterior thalamus would also be the result of altered circuit activity in the spatial processing network (*e.g.* the HPC, RSC, PoS and EC).

Information from the AT can enter the HPC via the EC (Taube, 2007). As the main input to the HPC, disruption to the EC would be likely cause downstream impact on spatial representations produced by the cognitive map. Although inactivation of the AT is known to cause severe disruption to grid cell firing in the EC (Winter et al., 2015), studies have shown that lesions to the ADN and PoS do not in fact cause considerable disturbance to hippocampal place cell activity (Calton et al., 2003) such that accurate cue control was retained and all characteristics except spatial coherence were unchanged. With regard to context processing, lesions of the EC also do not impair learning in contextual fear conditioning (Phillips and LeDoux, 1995). These studies suggest that any deficits in performance in this task, where information from the AT is disrupted, is unlikely to be dependent on the pathway via the EC.

The alternative route for information from the AT to reach the HPC comes via the fornix, the primary linkage between the hippocampus and subcortical areas (Phillips and LeDoux, 1995). In studies of context memory, neural patterns in the AT and RSC seem to be correlated with learning in particular contexts, with the suggestion that these patterns reflect the association of learned behaviour with the context that behaviour was learnt in (Freeman et al., 1996). Consistent with this idea, lesions of the fornix, that disconnect the hippocampus from the AT, disrupt these neural firing patterns and impair the ability to learn different behaviours in separate contexts (Smith et al., 2004). Fornix lesions also, pertinently to the present study, lead to severe impairments in the object-place-context experiment (Eacott and Norman, 2004), where the authors concluded that this impairment was produced by the need to combine object, place and context to resolve the task.

Taken together, the results of Experiment 3 support a conclusion that the AT, and likely by extension the head direction system, is critically involved in the resolution of directional ambiguity in the current task. Similarly to the task of Eacott and Norman (2004), this resolution relies on the generation of location-odour configurations, a process that appears to be impaired in instances when the hippocampus and the AT are disconnected by various means.

Experimental caveats

When working with diffusible agents, it is important to recognise the possibility of spread of the agent outside the region of interest. In this case, targeting the anterior thalamus with muscimol comes with the potential of post-infusion leakage into the third ventricle (as the anterior thalamus is bordered by the third ventricle on the dorsal side) or the hippocampus (as the implanted cannula terminates in the hippocampus). Muscimol infusions into the third ventricle reportedly result in short-lasting locomotor stimulation (5-10 minutes) followed by behavioural sedation or sleep for an average of ~100 minutes (Bagetta et al., 1987). Contrastingly, muscimol has been shown to induce locomotor hyperactivity when applied to the dorsal hippocampus (Bast and Feldon, 2003). The experimental paradigm described here included a 30 minute rest period post-infusion to assess any behavioural effects before the start of the experiment, and a habituation period lasting ~52 minutes before the probe trial. It is unlikely that the animals used in this experiment would have been able to perform the full paradigm without noticeable behavioural effects in the event of third ventricle or hippocampal leakage. However, any possible effect should be assessed by measuring the total path length of muscimol-treated animals throughout the experiment on the drug day and comparing this to the total path length of the sham animals.

It is also possible that the failure of the muscimol-treated animals to recognise displacement in the probe trial is due to a general spatial deficit, caused by disruption to the cognitive map. If this is the case, one would not expect the animal to be able to orient themselves within the environment, let alone resolve complex location-odour configurations. The additional trial for muscimol animals, where the spatial ambiguity is removed and the object replaced by a novel one, only serves to prove that the lack of exploration is not due to a general deficit of novelty per se and does not rely on the spatial location of the novel object. A potential control experiment to eliminate this hypothesis would be to displace one standard object to the centre of a single compartment, leaving the other in its usual position. If the animal explores the object in the centre of the compartment even though it is not novel, it will indicate that the animal

can indeed notice a spatial change but it would also not be informative about conditions of ambiguity or an ability to use odour to inform complex spatial configurations.

Figure 7.6: Potential experiment to eliminate the hypothesis of general spatial deficit. While the included test of non-spatial novelty serves to rule out any lack of response to novelty per se, it does not account for the possibility that the animals cannot recognise object displacement due to general spatial deficit. To account for this, the animals may be tested under a condition of spatial novelty – this involves using the same familiar objects, but moving the object to the middle of the compartment. Under this condition there remains no spatial ambiguity so if the animals were not generally impaired, they should detect an object in a novel location and preferentially explore this.

Chapter 8 - General Discussion

In everyday life, it is necessary to be able to resolve the ambiguity of visually similar spaces in order to navigate accurately to a destination or avoid becoming lost. Salient directional landmarks in the environment are useful as orienting features, but these landmarks can be unreliable if they have different meanings depending on the context in which they are encountered.

Previous studies have established that neurons of the hippocampal place system are able to the resolve spatial ambiguity presented by visually similar spaces using a variety of information such as colour (Spiers et al., 2015), directional offset (Grieves et al., 2016), and repeated experimenter-led processes such as the same starting compartment (Fuhs et al., 2005). Similarly, animals are known to be able to resolve spatial ambiguity using non-metric cues (Van Cauter et al., 2013; Davis et al., 2013; Eacott and Norman, 2004; Langston and Wood, 2010). This thesis sought to examine whether the spatial system in rats can use context to disambiguate visually similar environments that have different directional orientation. Specifically, whether this directional ambiguity can be resolved by animals using odour context, whether the hippocampal place system can also resolve this directional ambiguity, and whether these resolutions are mediated by the head direction system.

Experiment 1 confirmed that animals could solve a novel spontaneous recognition task in the two-compartment apparatus that involved creating configural representations of ‘where-which’: the animal was familiarised with a version of the environment where the placements of objects created a distinction between the two sides, then was exposed to a test trial where the objects were moved to create an environment only discriminable using odour. At test, the animals preferentially explored objects in a novel location-odour configuration rather than the familiar configuration, acknowledging that if presented with a lack of discriminable visual information then animals can use non-metric cues (in this case, odour) to orient themselves within an environment and organise a spatial representation based on landmarks (Anderson and Jeffery, 2003; Eacott and Norman, 2004; Langston and Wood, 2010; Maaswinkel and Whishaw, 1999).

Experiment 2 aimed to see whether the hippocampal place system could also solve the directional ambiguity afforded by the two-compartment apparatus, but additionally attempted to extend findings of head direction cell activity in this apparatus. A distinct population of head direction neurons in the retrosplenial cortex presented with bipolar tuning curves in the same two-compartment apparatus as this study. This bipolarity

suggested that, unlike 'classical' head direction cells co-recorded from the retrosplenial cortex and recorded in the ADN (by the author (D.Overington), see Appendix I) and PoS, these cells were not creating a consistent global representation of head direction in the apparatus by using the odour cues but instead responding only to local visual cues (Jacob et al., 2016). This result led to the proposal that there were conflicting streams of directional information that could be passed to the hippocampus: if the consistent stream was favoured, it was expected that directional information would provide the hippocampal place system with information that the spaces were separate and allow resolution of the two spaces; however, if there was processing of the bipolar input then it was expected that place fields would also anchor to more local cues and create 'flipped' representations of each compartment. It was shown here that hippocampal place cells could use odour context to inform representation of complex space and separate visually ambiguous compartments, and that this disambiguation presented as remapping (similarly to Anderson and Jeffery, 2003 and Spiers et al., 2015) or duplication (as in Skaggs and McNaughton, 1998; Fuhs et al., 2005; Spiers et al., 2015; and Grieves et al., 2016) between the two compartments with no sign of 'flipped' fields. The lack of any 'flipped' fields between compartments suggests that any purely local directional information that conflicts from the consistent global 'sense of direction' signal is filtered out by the time directional information arrives at the hippocampus.

Given the results of Jacob et al. (2016) and the results from Experiment 2, we then sought to determine whether the head direction system was involved in the task; if the head direction cells can process context then does the ability of the hippocampal place system to use context to separate similar spaces stem from directional activity? The results detailed in Experiment 3 show that when animals have a disruption to the head direction system (through an infusion of muscimol to the anterior thalamus), they do not show the same reaction to the spatial displacement in the test phase *i.e.* they do not increase their exploration of the displaced object. This suggests a lack of discrimination based on the contextual cues, leading to an inability to recognise which object has been displaced in the now visually symmetrical environment. However, drug animals can still react to basic novelty such that if the visual asymmetry is restored to a familiar configuration and a single object replaced with a novel one then exploration will increase for the replaced object. From this, we can propose that only the spatial aspect of solving the task is impaired. This result adds to current view that direction is very important to accurate spatial representation, and to the current literature that indicates that an increase in head direction information (*e.g.* adding an angle between

compartments) to experiments with visually similar spaces can make it easier for animals to discriminate between them (Fuhs et al., 2005; Grieves et al., 2016).

One factor that could affect the interpretation of these experiments as a whole is that the task differed between the two types of paradigm. While both paradigms did not use goal-directed tasks (the spontaneous recognition task and free foraging), the behavioural experiment required the use of objects that were not present in the electrophysiological recordings, while the animals participating in the electrophysiological recordings were uniquely trained to forage for rice. The rice itself could have also acted as an additional cue for the animal, due to the fact that the rice present in the vanilla compartment was also flavoured with vanilla. Thus, despite the fact that all three experiments in this thesis share the same recording environment (*i.e.* same odours, same apparatus), it is not possible to draw any conclusions regarding place cell activity within the behavioural experiments or any behaviour that could occur with a particular pattern of place cell activity.

The cognitive map, behaviour and spatial ambiguity

The guiding hypothesis of this thesis was that the two types of directional firing present in the retrosplenial cortex (Jacob et al., 2016) may have provided the animal with a way to resolve the directional ambiguity presented by the two-compartment context apparatus. From these studies, it seems clear that the retrosplenial cortex bipolar cells do not seem to provide input into the hippocampal place representation of this ambiguous space, and that the global head direction signal predominates at this level of coding in the brain. It has been hypothesised that the retrosplenial cortex bipolar cells instead perform a more complex integration of the two kinds of inputs (vision/odour and global head direction signal) and could act as a mediator between the two (Jacob et al., 2016), though it seems as though this level of processing may simply not be necessary for basic hippocampal spatial representation.

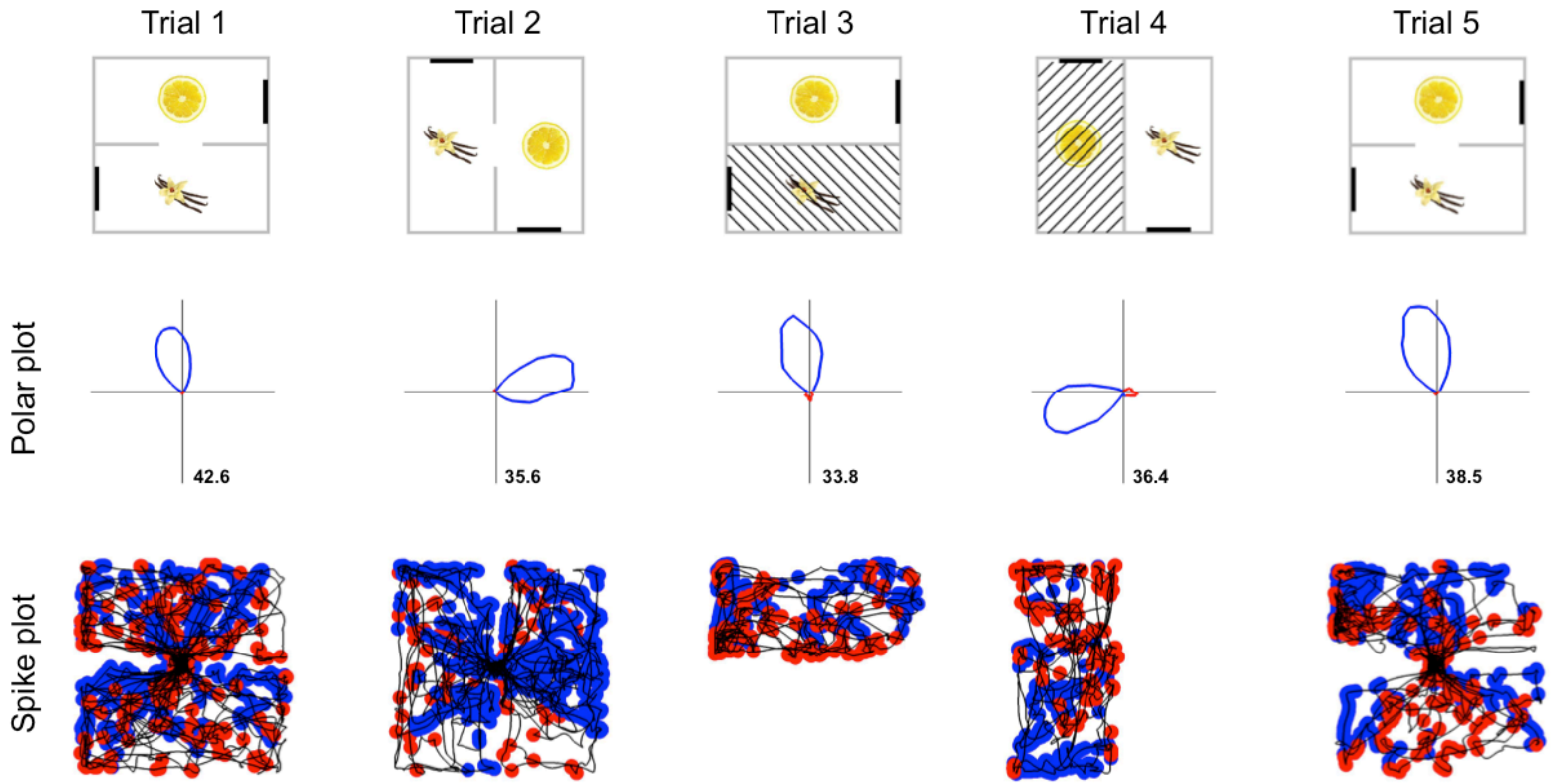
We hypothesised in Chapter 7 that the anterior thalamus, and thus the global directional signal, is critically involved in the resolution of the directional ambiguity of Experiments 1 and 3. Following disruption of the global head direction signal, as in Experiment 3, it may be the case that the bipolar signal persists and that this information is then used to drive spatial representation. If it is assumed that the head direction cell network, knowing it must use odour context to maintain a global signal, provides some contextual input to the hippocampus then it stands to reason that the persisting bipolar signal (with a lack of contextual information) could explain why the muscimol-treated animals in could not reconcile the location-odour configurations at

test. However, it is difficult to decouple behaviourally whether the lack of ability to solve the location-odour task is due to lack of proper hippocampal-thalamic communication or otherwise.

Future work aims to further dissect the relationship between the head direction system and the resolution of directional ambiguity in the two-compartment apparatus. Planned experiments will record hippocampal place cells and retrosplenial cortex neurons under anterior thalamus inactivation; these experiments will assess whether the local directional signal in the dysgranular RSC (Jacob et al., 2016) indeed persists under disruption of the global signal, if this signal influences place field activity under these conditions, and whether this signal can be used to resolve ambiguity. Optogenetic techniques will also be explored as a means of increasing the temporal and spatial precision of inactivating the anterior thalamus. In addition to this, another set of experiments will record hippocampal place cells in the behavioural paradigm to test whether there are any patterns of activity that correspond to recognition of spatial displacement.

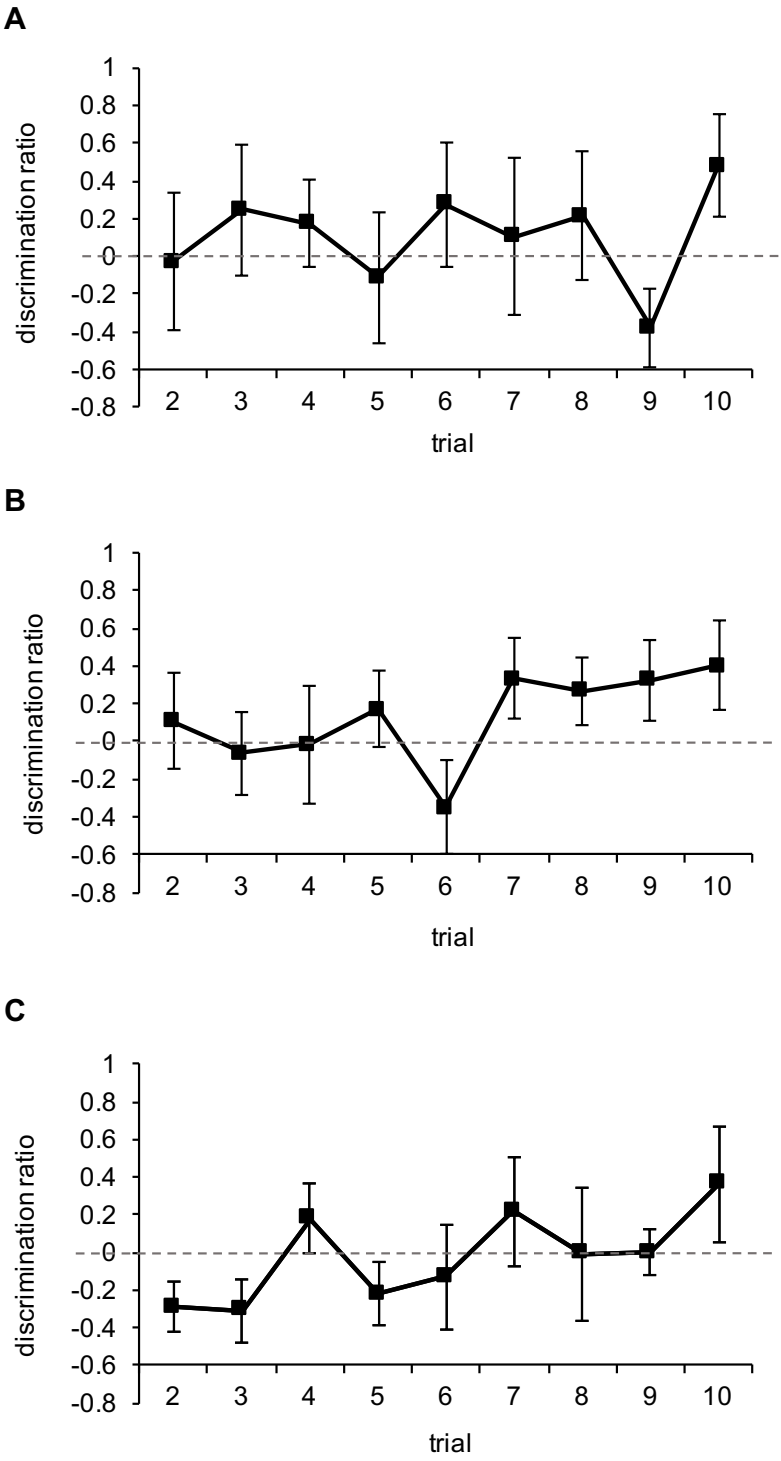
It is common for directional landmarks to be ambiguous in the absence of context, but salient and useful when context is available. For example, the Empire State Building is a useful orienting landmark provided the direction it is encountered from is known. This thesis has shown that rats can use non-metric odour cues as salient information to orient themselves in a visually cue-poor environment and resolve directional ambiguity of visual landmarks on both a cellular and behavioural level, and has also introduced new evidence to support the importance of the directional system to the formation of complete spatial representations under conditions of ambiguity. Together, this thesis adds to the wealth of studies that link the activity of the hippocampal place and head direction systems to active navigation and spatial learning, lending more evidence that animals do indeed possess a flexible neural representation of space and context and a so-called cognitive map (O'Keefe and Nadel, 1978; Tolman, 1948).

Appendix I



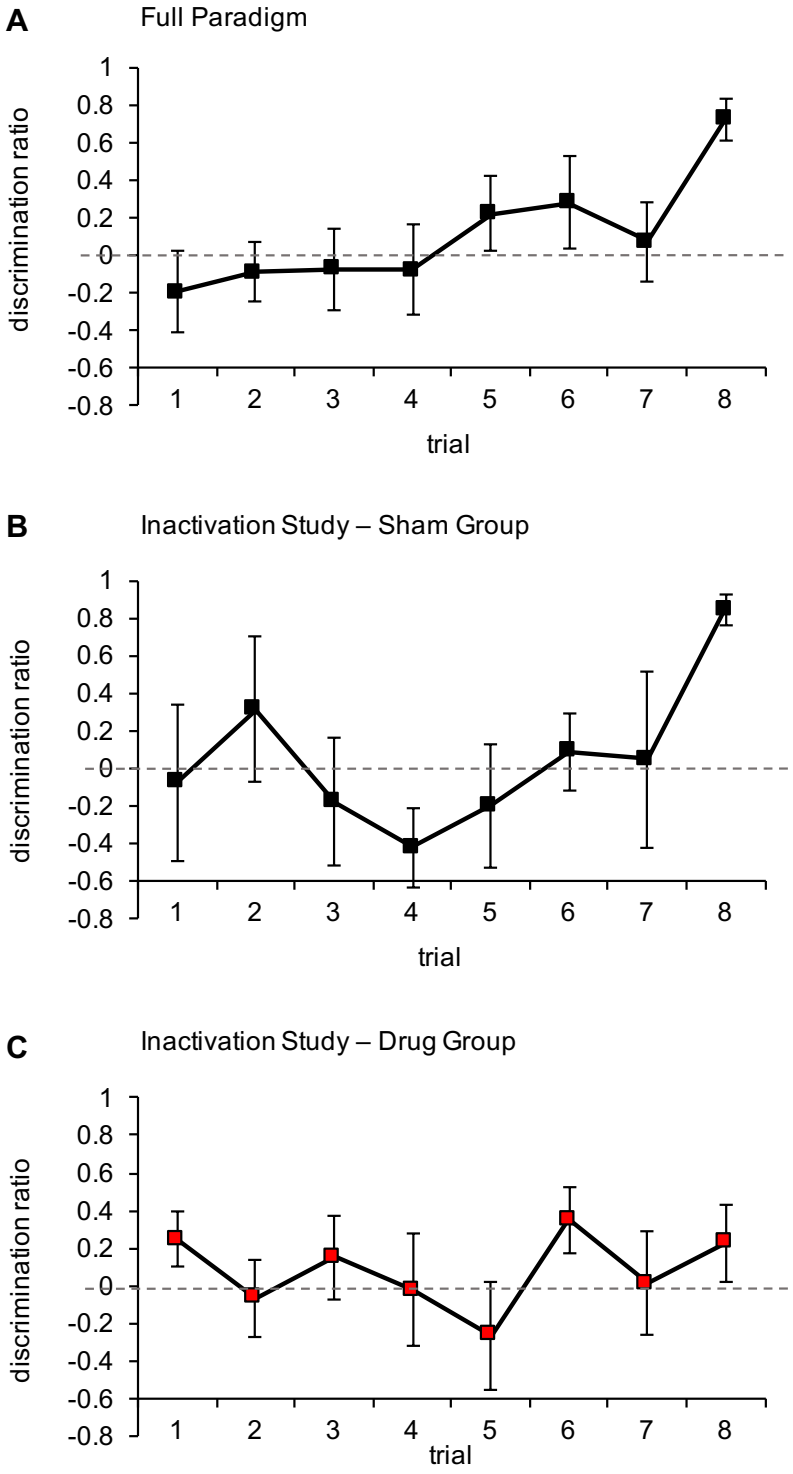
Appendix 1: Representative ADN head direction cell recorded in the two-compartment box. This cell maintains a consistent global heading direction over all 5 trials, reflecting a use of context to resolve directional ambiguity (as in Jacob et al., *in press*). Peak firing rates (Hz) for the cell are indicated at the bottom right of the polar plot. Red and blue colours in the spike plot correspond to spikes fired in the peak firing direction (blue) and spikes fired in a direction 180° opposite (red); the lack of a red lobe in the polar plot indicates that this cell was maximally tuned to its peak firing direction, unlike a bipolar cell found in the dysgranular retrosplenial cortex (Jacob et al., *in press*).

Appendix II



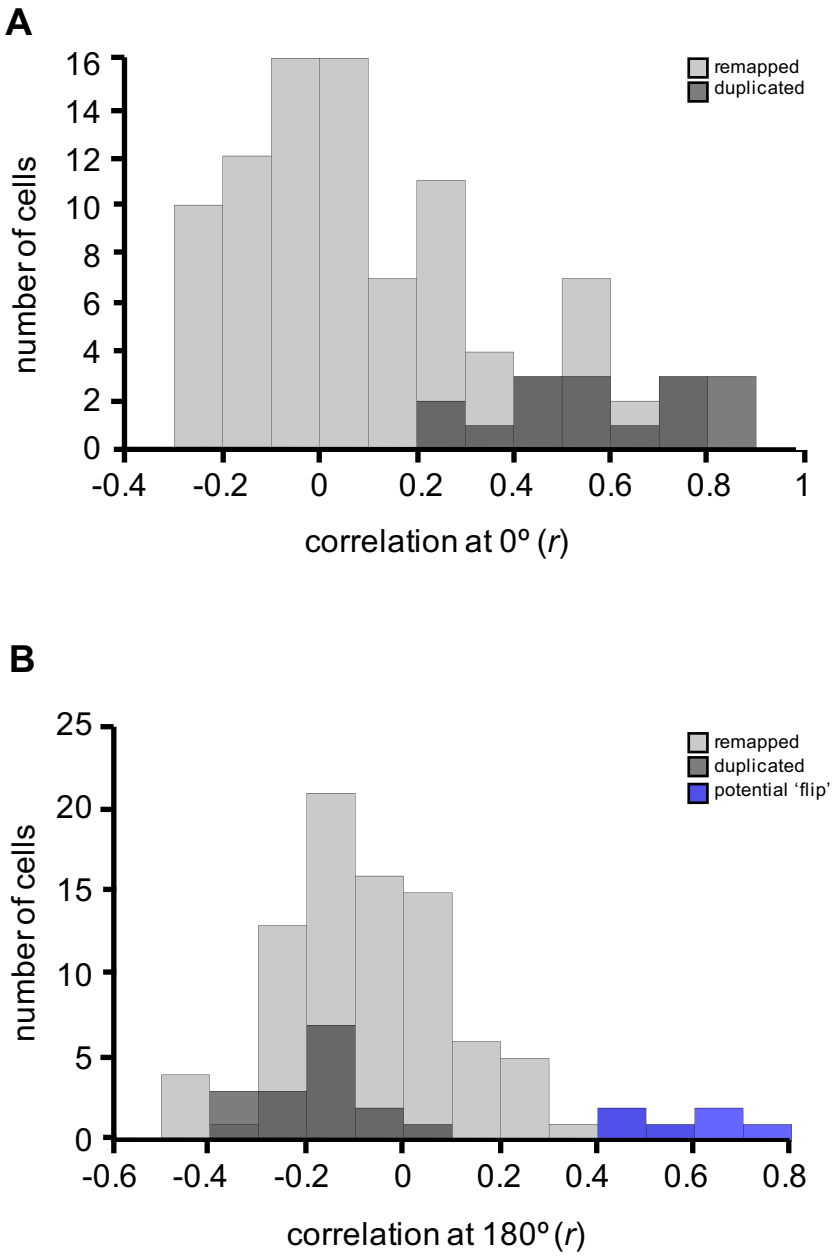
Appendix 2: D2 discrimination analysis of the pilot experiments. (A) Pilot 1; there were no significant differences in discrimination index over the experiment, both the habituation phase and probe trial (one-way ANOVA [$F(8,45) = 0.635$, $p = .744$]. (B) Pilot 2; there were no significant differences in discrimination index over the experiment (one-way ANOVA, [$F(8,45) = 1.069$, $p = .402$]. (C) Pilot 3; there were no significant differences in discrimination index over the experiment (one-way ANOVA, [$F(8,45) = 1.015$, $p = .438$]).

Appendix III



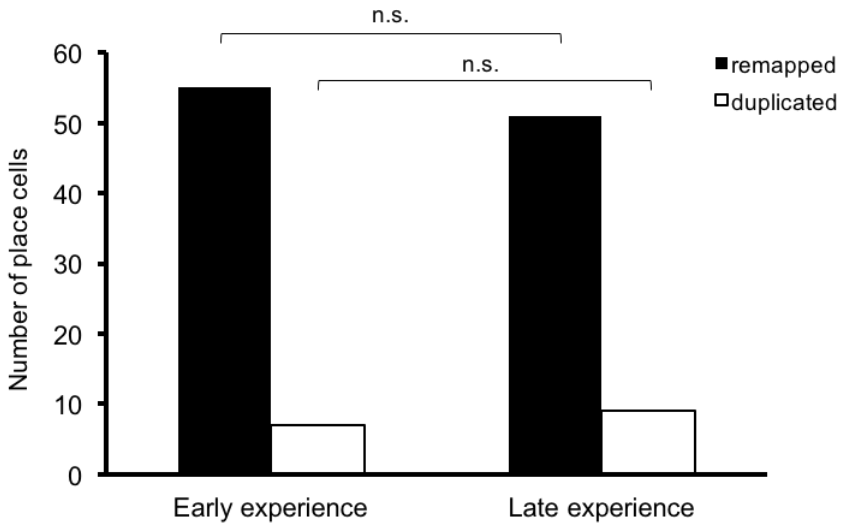
Appendix 3: D2 discrimination index of the full behavioural paradigm and inactivation experiments. (A) Full paradigm; no significant difference was observed over the experiment (including the probe trial) (one-way ANOVA, [$F(7,79) = 2.117$, $p = .052$]. (B) Inactivation Study – Sham Group; there was a significant difference in discrimination index over the experiment (one-way ANOVA, [$F(7,79) = 2.796$, $p = .014$]. (C) Inactivation Study – Drug Group; no significant difference was observed over the experiment (one-way ANOVA, [$F(7,79) = 0.750$, $p = .631$]).

Appendix IV



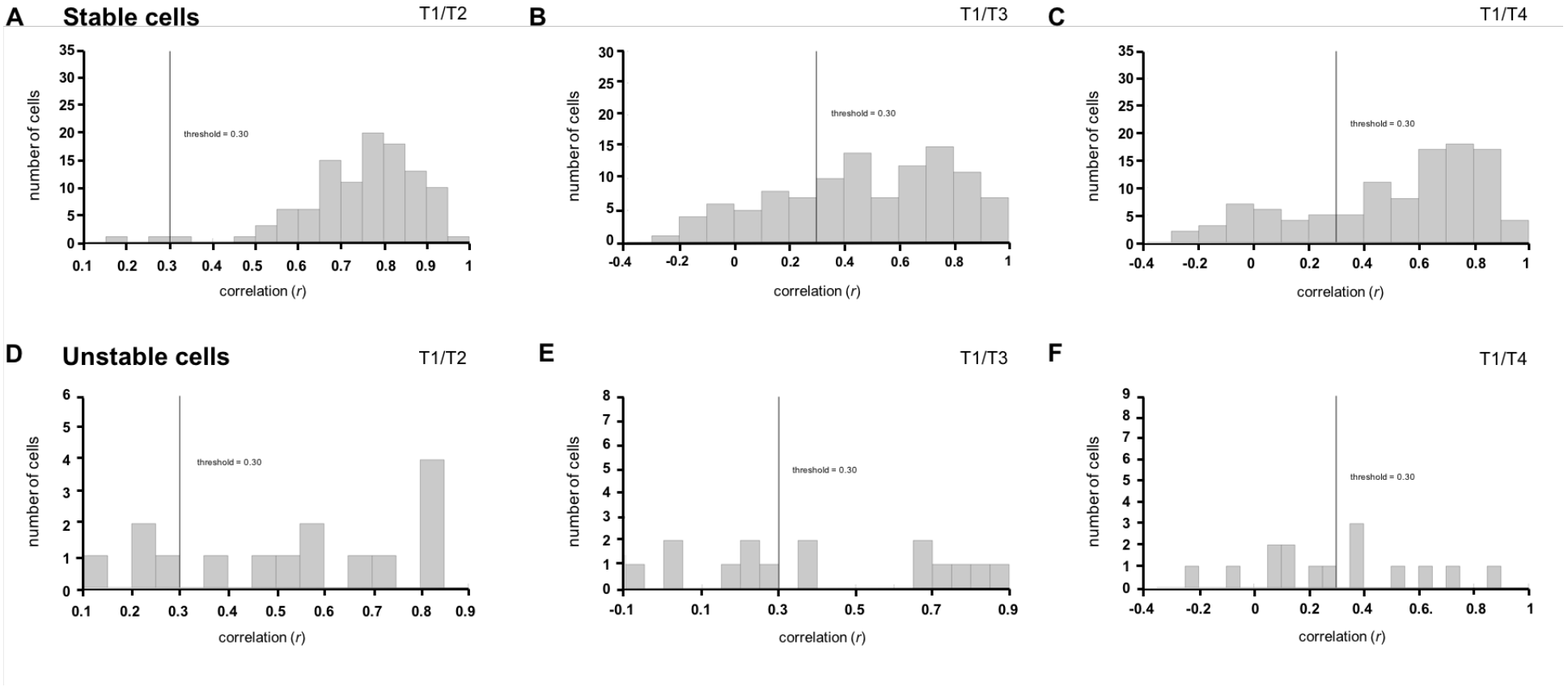
Appendix 4: Histograms of pixelwise correlations within maps at 0 and 180°. (A) Within map correlations (lemon versus vanilla compartment) at 0° - remapped cells have a range of correlation values, with a peak around 0°, while duplicated cells have a much smaller range of correlation values that tend towards high *r* values. (B) Within map correlations at 180°, 'remapped' and 'duplicated' populations are the same as those in (A) - as expected, 'remapped' cells had a large range of correlation values (peaking around *r*=-0.1) while 'duplicated' cells had low correlation values at 180°. A subsection of 'remapped' cells had high correlation values at 180, which would theoretically indicated 'flip' activity (coloured blue); on further investigation, these cells did not have flip activity but instead usually presented with a central 'door field' that biased the correlation (Figure 6.6).

Appendix V



Appendix 5: Full data set of recorded place cells stratified by experience. There is no significant difference in the proportion of 'remapped' and 'duplicated' place cells when separated by experience (early experience, z=0.388, p > 0.05.; late experience, z=-0.5, p > 0.05). Early experience was defined as three or less exposures to the context box per animal, and late experience were exposures 4 and above.

Appendix VI



Appendix 6: Single compartment analysis - histograms of between-trial correlations. The data presented here are also presented in Figure 6.8; remapping threshold of 0.3 is marked with solid black line. (A) T1/T2: the closed door trials retain the same spatial representation as trial 1. (B,C) T1/T3 and T1/T4: the closed door trials, on average, represent the same spatial representation as trial 1 but the range of these correlation values is larger. This is likely to be due to local remapping (field generation) following introduction of barriers in Alvernhe et al., 2008, 2011. (D) T1/T2 unstable cells: on average, the closed door trials maintain a correlation above the remapping threshold, suggesting maintenance of the spatial representation. (E,F) T1/T3 and T1/T4: the closed door trials have a large range of correlation values with no clear peak.

References

Aggleton, J.P., Hunt, P.R., Nagle, S., and Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. Behav. Brain Res. *81*, 189–198.

Ainge, J.A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., and Wood, E.R. (2006). The role of the hippocampus in object recognition in rats: Examination of the influence of task parameters and lesion size. Behav. Brain Res. *167*, 183–195.

Ainge, J.A., Meer, M.A.A. Van Der, Langston, R.F., and Wood, E.R. (2007). Exploring the Role of Context-Dependent Hippocampal Activity in Spatial Alternation Behavior. *1002*, 988–1002.

Alvernhe, A., Van Cauter, T., Save, E., and Poucet, B. (2008). Different CA1 and CA3 representations of novel routes in a shortcut situation. J. Neurosci. *28*, 7324–7333.

Alvernhe, A., Save, E., and Poucet, B. (2011). Local remapping of place cell firing in the Tolman detour task. Eur. J. Neurosci. *33*, 1696–1705.

Amaral, D.G., and Lavenex, P. (2007). Hippocampal neuroanatomy. In The Hippocampus Book, D.G. Amaral, P. Andersen, T. Bliss, R.G.M. Morris, and J. O'Keefe, eds. pp. 37–114.

Amaral, D.G., and Witter, M.P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience *31*, 571–591.

Anderson, M.I., and Jeffery, K.J. (2003). Heterogeneous modulation of place cell firing by changes in context. J. Neurosci. *23*, 8827–8835.

Anderson, M.I., Killing, S., Morris, C., O'Donoghue, A., Onyiagha, D., Stevenson, R., Verriotis, M., and Jeffery, K.J. (2006). Behavioral correlates of the distributed coding of spatial context. Hippocampus *16*, 730–742.

André, M.A.E., and Manahan-Vaughan, D. (2013). Spatial olfactory learning facilitates long-term depression in the hippocampus. Hippocampus *23*, 963–968.

Bagetta, G., Sakurada, S., Corasaniti, M.T., Froio, F., and Nisticò, G. (1987). Behavioural and electrocortical changes induced by muscimol in rats withdrawn from chronic treatment with diazepam. Neuropharmacology *26*, 725–730.

Barry, C., Lever, C., Hayman, R., Hartley, T., Burton, S., O'Keefe, J., Jeffery, K., and

Burgess, N. (2006). The boundary vector cell model of place cell firing and spatial memory. Rev. Neurosci. *17*, 71–97.

Barry, C., Hayman, R., Burgess, N., and Jeffery, K.J. (2007). Experience-dependent rescaling of entorhinal grids. Nat. Neurosci. *10*, 682–684.

Barry, C., Ginzberg, L.L., O'Keefe, J., and Burgess, N. (2012). Grid cell firing patterns signal environmental novelty by expansion. Proc. Natl. Acad. Sci. U. S. A. *109*, 17687–17692.

Bassett, J.P., and Taube, J.S. (2005). Head direction signal generation: Ascending and descending information streams. In Head Direction Cells and the Neural Mechanisms of Spatial Orientation, S.I. Weiner, and J.S. Taube, eds. (MIT Press, Cambridge, MA), pp. 83–109.

Bassett, J.P., Tullman, M.L., and Taube, J.S. (2007). Lesions of the tegmentomammillary circuit in the head direction system disrupt the head direction signal in the anterior thalamus. J. Neurosci. *27*, 7564–7577.

Bast, T., and Feldon, J. (2003). Hippocampal modulation of sensorimotor processes. Prog. Neurobiol. *70*, 319–345.

Bingman, V.P., Siegel, J.J., Gagliardo, A., and Erichsen, J.T. (2006). Representing the richness of avian spatial cognition: properties of a lateralized homing pigeon hippocampus. Rev. Neurosci. *17*, 17–28.

Bjerknes, T.L., Moser, E.I., and Moser, M.-B. (2014). Representation of geometric borders in the developing rat. Neuron *82*, 71–78.

Blair, H.T., Cho, J., and Sharp, P.E. (1998). Role of the lateral mammillary nucleus in the rat head direction circuit: a combined single unit recording and lesion study. Neuron *21*, 1387–1397.

Blair, H.T., Cho, J., and Sharp, P.E. (1999). The anterior thalamic head-direction signal is abolished by bilateral but not unilateral lesions of the lateral mammillary nucleus. J. Neurosci. *19*, 6673–6683.

Boccara, C.N., Sargolini, F., Thoresen, V.H., Solstad, T., Witter, M.P., Moser, E.I., and Moser, M.-B. (2010). Grid cells in pre- and parasubiculum. Nat. Neurosci. *13*, 987–994.

Bonnevie, T., Dunn, B., Fyhn, M., Hafting, T., Derdikman, D., Kubie, J.L., Roudi, Y., Moser, E.I., and Moser, M.-B. (2013). Grid cells require excitatory drive from the hippocampus. Nat. Neurosci. *16*, 309–317.

Bostock, E., Muller, R.U., and Kubie, J.L. (1991). Experience-dependent modifications of hippocampal place cell firing. Hippocampus *1*, 193–205.

Brun, V.H., Solstad, T., Kjelstrup, K.B., Fyhn, M., Witter, M.P., Moser, E.I., and Moser, M.-B. (2008). Progressive increase in grid scale from dorsal to ventral medial entorhinal cortex. Hippocampus *18*, 1200–1212.

Burgalossi, A., Herfst, L., von Heimendahl, M., Förste, H., Haskic, K., Schmidt, M., and Brecht, M. (2011). Microcircuits of functionally identified neurons in the rat medial entorhinal cortex. Neuron *70*, 773–786.

Burgess, N., Jackson, A., Hartley, T., and O'Keefe, J. (2000). Predictions derived from modelling the hippocampal role in navigation. Biol. Cybern. *83*, 301–312.

Burwell, R.D. (2000). The parahippocampal region: corticocortical connectivity. Ann. N. Y. Acad. Sci. *911*, 25–42.

Burwell, R.D. (2004). Perirhinal and Postrhinal Contributions to Remote Memory for Context. J. Neurosci. *24*, 11023–11028.

Burwell, R.D., and Agster, K.L. (2008). Anatomy of the Hippocampus and the Declarative Memory System. In Learning and Memory: A Comprehensive Reference., J.H. Byrne, ed. (Academic Press, Oxford), pp. 47–66.

Butterly, D.A., Petroccione, M.A., and Smith, D.M. (2012). Hippocampal context processing is critical for interference free recall of odor memories in rats. Hippocampus *22*, 906–913.

Calton, J.L., Stackman, R.W., Goodridge, J.P., Archey, W.B., Dudchenko, P.A., and Taube, J.S. (2003). Hippocampal place cell instability after lesions of the head direction cell network. J. Neurosci. *23*, 9719–9731.

Canto, C.B., Wouterlood, F.G., and Witter, M.P. (2008). What does the anatomical organization of the entorhinal cortex tell us? Neural Plast. *2008*, 381243.

Van Cauter, T., Camon, J., Alvernhe, A., Elduayen, C., Sargolini, F., and Save, E. (2013). Distinct roles of medial and lateral entorhinal cortex in spatial cognition. Cereb. Cortex *23*, 451–459.

Chen, L.L., Lin, L.H., Green, E.J., Barnes, C.A., and McNaughton, B.L. (1994). Head-direction cells in the rat posterior cortex. I. Anatomical distribution and behavioral modulation. Exp. Brain Res. *101*, 8–23.

Chevaleyre, V., and Siegelbaum, S.A. (2010). Strong CA2 pyramidal neuron synapses

define a powerful disynaptic cortico-hippocampal loop. Neuron *66*, 560–572.

Cho, J., and Sharp, P.E. (2001). Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. Behav. Neurosci. *115*, 3–25.

Clark, B.J., and Taube, J.S. (2011). Intact landmark control and angular path integration by head direction cells in the anterodorsal thalamus after lesions of the medial entorhinal cortex. Hippocampus *21*, 767–782.

Clark, B.J., and Taube, J.S. (2012). Vestibular and attractor network basis of the head direction cell signal in subcortical circuits. Front. Neural Circuits *6*, 1–12.

Clark, B.J., Bassett, J.P., Wang, S.S., and Taube, J.S. (2010). Impaired head direction cell representation in the anterodorsal thalamus after lesions of the retrosplenial cortex. J. Neurosci. *30*, 5289–5302.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (L. Erlbaum Associates).

Corkin, S. (2002). What's new with the amnesic patient H.M.? Nat. Rev. Neurosci. *3*, 153–160.

Cressant, A., Muller, R.U., and Poucet, B. (1997). Failure of centrally placed objects to control the firing fields of hippocampal place cells. J. Neurosci. *17*, 2531–2542.

Davis, K.E., Easton, A., Eacott, M.J., and Gigg, J. (2013). Episodic-like memory for what-where-which occasion is selectively impaired in the 3xTgAD mouse model of Alzheimer's disease. J. Alzheimer's Dis. *33*, 681–698.

Derdikman, D., Whitlock, J.R., Tsao, A., Fyhn, M., Hafting, T., Moser, M.-B., and Moser, E.I. (2009). Fragmentation of grid cell maps in a multicompartment environment. Nat. Neurosci. *12*, 1325–1332.

Deshmukh, S.S., and Knierim, J.J. (2011). Representation of non-spatial and spatial information in the lateral entorhinal cortex. Front. Behav. Neurosci. *5*, 69.

Dickson, C.T., Magistretti, J., Shalinsky, M., Hamam, B., and Alonso, A. (2000). Oscillatory activity in entorhinal neurons and circuits. Mechanisms and function. Ann. N. Y. Acad. Sci. *911*, 127–150.

Doeller, C.F., Barry, C., and Burgess, N. (2010). Evidence for grid cells in a human memory network. Nature *463*, 657–661.

Eacott, M.J., and Norman, G. (2004). Integrated memory for object, place, and context

in rats: a possible model of episodic-like memory? J. Neurosci. *24*, 1948–1953.

Easton, A., Fitchett, A.E., Eacott, M.J., and Baxter, M.G. (2010). Medial septal cholinergic neurons are necessary for context-place memory but not episodic-like memory. Hippocampus *21*, n/a-n/a.

Ekstrom, A.D., Kahana, M.J., Caplan, J.B., Fields, T.A., Isham, E.A., Newman, E.L., and Fried, I. (2003). Cellular networks underlying human spatial navigation. Nature *425*, 184–188.

Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res. *31*, 47–59.

Etienne, A.S., and Jeffery, K.J. (2004). Path integration in mammals. Hippocampus *14*, 180–192.

Fenton, A.A., Lytton, W.W., Barry, J.M., Lenck-Santini, P.-P., Zinyuk, L.E., Kubík, S., Bures, J., Poucet, B., Muller, R.U., and Olypher, A. V (2010). Attention-like modulation of hippocampus place cell discharge. J. Neurosci. *30*, 4613–4625.

Frank, L.M., Brown, E.N., and Wilson, M. (2000). Trajectory encoding in the hippocampus and entorhinal cortex. Neuron *27*, 169–178.

Freeman, J.H., Cuppernell, C., Flannery, K., and Gabriel, M. (1996). Context-specific multi-site cingulate cortical, limbic thalamic, and hippocampal neuronal activity during concurrent discriminative approach and avoidance training in rabbits. J. Neurosci. *16*, 1538–1549.

Fuhs, M.C., and Touretzky, D.S. (2006). A spin glass model of path integration in rat medial entorhinal cortex. J. Neurosci. *26*, 4266–4276.

Fuhs, M.C., VanRhoads, S.R., Casale, A.E., Mcnaughton, B., and Touretzky, D.S. (2005). Influence of path integration versus environmental orientation on place cell remapping between visually identical environments. J. Neurophysiol. 2603–2616.

Furtak, S.C., Wei, S.-M., Agster, K.L., and Burwell, R.D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. Hippocampus *17*, 709–722.

Fyhn, M., Molden, S., Witter, M.P., Moser, E.I., and Moser, M.-B. (2004). Spatial representation in the entorhinal cortex. Science *305*, 1258–1264.

Fyhn, M., Hafting, T., Treves, A., Moser, M.-B., and Moser, E.I. (2007). Hippocampal remapping and grid realignment in entorhinal cortex. Nature *446*, 190–194.

Fyhn, M., Hafting, T., Witter, M.P., Moser, E.I., and Moser, M.-B. (2008). Grid cells in mice. Hippocampus *18*, 1230–1238.

Good, M.A., Barnes, P., Staal, V., Mcgregor, A., and Honey, R.C. (2007). Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. Behav. Neurosci. *121*, 218–223.

Goodridge, J.P., and Taube, J.S. (1997). Interaction between the postsubiculum and anterior thalamus in the generation of head direction cell activity. J. Neurosci. *17*, 9315–9330.

Goodridge, J.P., Dudchenko, P.A., Worboys, K.A., Golob, E.J., and Taube, J.S. (1998). Cue control and head direction cells. Behav. Neurosci. *112*, 749–761.

Gothard, K.M., Skaggs, W.E., and McNaughton, B.L. (1996). Dynamics of mismatch correction in the hippocampal ensemble code for space: interaction between path integration and environmental cues. J. Neurosci. *16*, 8027–8040.

Grieves, R.M., Jenkins, B.W., Harland, B.C., Wood, E.R., and Dudchenko, P.A. (2016). Place field repetition and spatial learning in a multicompartment environment. Hippocampus *26*, 118–134.

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., and Moser, E.I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature *436*, 801–806.

Hargreaves, E.L., Rao, G., Lee, I., and Knierim, J.J. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science (80-.). *308*, 1792–1794.

Hartley, T., Burgess, N., Lever, C., Cacucci, F., and O'Keefe, J. (2000). Modeling place fields in terms of the cortical inputs to the hippocampus. Hippocampus *10*, 369–379.

Hayman, R.M.A., Chakraborty, S., Anderson, M.I., and Jeffery, K.J. (2003). Context-specific acquisition of location discrimination by hippocampal place cells. Eur. J. Neurosci. *18*, 2825–2834.

Henze, D.A., Borhegyi, Z., Csicsvari, J., Mamiya, A., Harris, K.D., and Buzsáki, G. (2000). Intracellular features predicted by extracellular recordings in the hippocampus in vivo. J. Neurophysiol. *84*, 390–400.

Hetherington, P.A., and Shapiro, M.L. (1997). Hippocampal place fields are altered by the removal of single visual cues in a distance-dependent manner. Behav. Neurosci. *111*, 20–34.

Hill, A.J., and Best, P.J. (1981). Effects of deafness and blindness on the spatial correlates of hippocampal unit activity in the rat. Exp. Neurol. *217*, 204–217.

Huxter, J., Burgess, N., and O'Keefe, J. (2003). Independent rate and temporal coding in hippocampal pyramidal cells. Nature *425*, 828–832.

Jacob, P.-Y., Casali, G., Spieser, L., Page, H., Overington, D., and Jeffery, K. (2016). Uncoupling of dysgranular retrosplenial "head direction" cells from the global head direction network. Nat. Neurosci. *20*, 173–175.

Jeffery, K.J. (2007). Self-localization and the entorhinal-hippocampal system. Curr. Opin. Neurobiol. *17*, 684–691.

Jeffery, K.J., and O'Keefe, J.M. (1999). Learned interaction of visual and idiothetic cues in the control of place field orientation. Exp. Brain Res. *127*, 151–161.

Jensen, O., and Lisman, J.E. (2000). Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding. J. Neurophysiol. *83*, 2602–2609.

Jonckheere, A.R., and Bower, G.H. (1967). Non-parametric tests for learning data. Br. J. Math. Stat. Psychol. *20*, 163–186.

Jung, M.W., Wiener, S.I., and McNaughton, B.L. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. J. Neurosci. *14*, 7347–7356.

Kim, J.J., and Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. Science *256*, 675–677.

Kim, S., Sapiurka, M., Clark, R.E., and Squire, L.R. (2013). Contrasting effects on path integration after hippocampal damage in humans and rats. Proc. Natl. Acad. Sci. U. S. A. *110*, 4732–4737.

Kjelstrup, K.B., Solstad, T., Brun, V.H., Hafting, T., Leutgeb, S., Witter, M.P., Moser, E.I., and Moser, M.-B. (2008). Finite scale of spatial representation in the hippocampus. Science (80-.). *321*, 140–143.

Knierim, J.J. (2006). Neural representations of location outside the hippocampus. Learn. Mem. *13*, 405–415.

Knierim, J.J., Kudrimoti, H.S., and McNaughton, B.L. (1995). Place cells, head direction cells, and the learning of landmark stability. J. Neurosci. *15*, 1648–1659.

Knight, R., Piette, C.E., Page, H., Walters, D., Marozzi, E., Nardini, M., Stringer, S., and Jeffery, K.J. (2014). Weighted cue integration in the rodent head direction system. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *369*, 20120512.

Köhler, C. (1986). Cytochemical architecture of the entorhinal area. Adv. Exp. Med. Biol. *203*, 83–98.

Kropff, E., Carmichael, J.E., Moser, M.-B., and Moser, E.I. (2015). Speed cells in the medial entorhinal cortex. Nature *523*, 419–424.

Krupic, J., Bauza, M., Burton, S., Barry, C., and O'Keefe, J. (2015). Grid cell symmetry is shaped by environmental geometry. Nature *518*, 232–235.

Kumar, S.S., Jin, X., Buckmaster, P.S., and Huguenard, J.R. (2007). Recurrent circuits in layer II of medial entorhinal cortex in a model of temporal lobe epilepsy. J. Neurosci. *27*, 1239–1246.

Langston, R.F., and Wood, E.R. (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. Hippocampus *20*, 1139–1153.

Law, L.M., and Smith, D.M. (2012). The anterior thalamus is critical for overcoming interference in a context-dependent odor discrimination task. Behav. Neurosci. *125*, 710–719.

Lenck-Santini, P.-P., Rivard, B., Muller, R.U., and Poucet, B. (2005). Study of CA1 place cell activity and exploratory behavior following spatial and nonspatial changes in the environment. Hippocampus *15*, 356–369.

Leutgeb, S., Leutgeb, J.K., Barnes, C.A., Moser, E.I., McNaughton, B.L., and Moser, M.-B. (2005). Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science *309*, 619–623.

Lever, C., Wills, T., Cacucci, F., Burgess, N., and O'Keefe, J. (2002). Long-term plasticity in hippocampal place-cell representation of environmental geometry. Nature *416*, 90–94.

Lever, C., Burton, S., Jeewajee, A., O'Keefe, J., and Burgess, N. (2009). Boundary vector cells in the subiculum of the hippocampal formation. J. Neurosci. *29*, 9771–9777.

Li, X.G., Somogyi, P., Ylinen, A., and Buzsáki, G. (1994). The hippocampal CA3 network: an in vivo intracellular labeling study. J. Comp. Neurol. *339*, 181–208.

Lu, L., Igarashi, K.M., Witter, M.P., Moser, E.I., and Moser, M.-B. (2015). Topography of place maps along the CA3-to-CA2 axis of the hippocampus. Neuron *87*, 1078–1092.

Maaswinkel, H., and Whishaw, I.Q. (1999). Homing with locale, taxon, and dead reckoning strategies by foraging rats: sensory hierarchy in spatial navigation. Behav. Brain Res. *99*, 143–152.

Maaswinkel, H., Jarrard, L.E., and Whishaw, I.Q. (1999). Hippocampectomized rats are impaired in homing by path integration. Hippocampus *9*, 553–561.

Majchrzak, M., and Di Scala, G. (2000). GABA and muscimol as reversible inactivation tools in learning and memory. Neural Plast. *7*, 19–29.

Mankin, E.A., Diehl, G.W., Sparks, F.T., Leutgeb, S., and Leutgeb, J.K. (2015). Hippocampal CA2 activity patterns change over time to a larger extent than between spatial contexts. Neuron *85*, 190–201.

Manns, J.R., and Eichenbaum, H. (2006). Evolution of declarative memory. Hippocampus *16*, 795–808.

Marozzi, E., and Jeffery, K.J. (2012). Place, space and memory cells. Curr. Biol. *22*, R939–R942.

Marozzi, E., Ginzberg, L.L., Alenda, A., and Jeffery, K.J. (2015). Purely translational realignment in grid cell firing patterns following non-metric context change. Cereb. Cortex *25*, 4619–4627.

McNaughton, B.L., Barnes, C.A., and O'Keefe, J. (1983). The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. Exp. Brain Res. *52*, 41–49.

McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., and Moser, M.-B. (2006). Path integration and the neural basis of the "cognitive map". Nat. Rev. Neurosci. *7*, 663–678.

Mercer, A., Trigg, H.L., and Thomson, A.M. (2007). Characterization of neurons in the CA2 subfield of the adult rat hippocampus. J. Neurosci. *27*, 7329–7338.

Mittelstaedt, M.-L., and Mittelstaedt, H. (1980). Homing by path integration in a mammal. Nature *67*, 566–567.

Moita, M.A.P., Rosis, S., Zhou, Y., LeDoux, J.E., and Blair, H.T. (2004). Putting fear in its place: remapping of hippocampal place cells during fear conditioning. J. Neurosci. *24*, 7015–7023.

Moser, E.I., Kropff, E., and Moser, M.-B. (2008). Place cells, grid cells, and the brain's spatial representation system. Annu. Rev. Neurosci. *31*, 69–89.

Moser, E.I., Roudi, Y., Witter, M.P., Kentros, C., Bonhoeffer, T., and Moser, M.-B. (2014). Grid cells and cortical representation. Nat. Rev. Neurosci. *15*, 466–481.

Muller, R. (1996). A quarter of a century of place cells. Neuron *17*, 813–822.

Muller, R.U., and Kubie, J.L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J. Neurosci. *7*, 1951–1968.

Muller, R.U., Kubie, J.L., and Ranck, J.B. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J. Neurosci. *7*, 1935–1950.

Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., and Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learn. Mem. *9*, 49–57.

Neves, G., Cooke, S.F., and Bliss, T.V.P. (2008). Synaptic plasticity, memory and the hippocampus - a neural network aproach to causality. Nat. Rev. Neurosci. *9*, 65–75.

Norman, G., and Eacott, M.J. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. Behav. Neurosci. *119*, 557–566.

O'Keefe, J., and Burgess, N. (1996). Geometric determinants of the place fields of hippocampal neurons. Nature *381*, 425–428.

O'Keefe, J., and Burgess, N. (2005). Dual phase and rate coding in hippocampal place cells: theoretical significance and relationship to entorhinal grid cells. Hippocampus *15*, 853–866.

O'Keefe, J., and Conway, D.H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. Exp. Brain Res. *31*, 573–590.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res. *34*, 171–175.

O'Keefe, J., and Nadel, L. (1978). The hippocampus as a cognitive map (Oxford University Press).

O'Keefe, J., and Recce, M.L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus *3*, 317–330.

O'Keefe, J., and Speakman, A. (1987). Single unit activity in the rat hippocampus

during a spatial memory task. Exp. Brain Res. *68*, 1–27.

O'Reilly, R.C., and Rudy, J.W. (2000). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychol. Rev. *108*, 311–345.

Paxinos, G., and Watson, C. (1982). The Rat Brain in Stereotaxic Coordinates (Academic Press).

Paz-Villagran, V., Save, E., and Poucet, B. (2004). Independent coding of connected environments by place cells. Eur. J. Neurosci. *20*, 1379–1390.

Phillips, R.G., and LeDoux, J.E. (1995). Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning. J. Neurosci. *15*, 5308–5315.

Pocock, S.J. (2006). The simplest statistical test: how to check for a difference between treatments. Br. Med. J. *332*, 1256–1258.

Quirk, G.J., Muller, R.U., and Kubie, J.L. (1990). The firing of hippocampal place cells in the dark depends on the rat's recent experience. J. Neurosci. *10*, 2008–2017.

Ranck, J.B. (1973). Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. Exp. Neurol. *41*, 461–531.

Ranck, J.B. (1984). Head-direction cells in the deep cell layers of dorsal presubiculum in freely moving rats. Soc. Neurosci. Abs. *10*, 599.

Raudies, F., and Hasselmo, M.E. (2012). Modeling boundary vector cell firing given optic flow as a cue. PLoS Comput. Biol. *8*, e1002553.

Recce, M.L., and O'Keefe, J.M. (1989). The tetrode: a new technique for multi-unit extracellular recording. Soc. Neurosci. Abs. *15*, 1250.

Rolls, E.T., Miyashita, Y., Cahusac, P.M., Kesner, R.P., Niki, H., Feigenbaum, J.D., and Bach, L. (1989). Hippocampal neurons in the monkey with activity related to the place in which a stimulus is shown. J. Neurosci. *9*, 1835–1845.

Rosenthal, R., and Fode, K.L. (2007). The effect of experimenter bias on the performance of the albino rat. Behav. Sci. *8*, 183–189.

Rudy, J.W. (2009). Context representations, context functions, and the parahippocampal-hippocampal system. Learn. Mem. *16*, 573–585.

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B.L., Witter, M.P., Moser, M.-B., and Moser, E.I. (2006). Conjunctive representation of position, direction, and velocity in

entorhinal cortex. Science (80-.). *312*, 758–762.

Save, E., Poucet, B., Foreman, N., and Buhot, M.C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. Behav. Neurosci. *106*, 447–456.

Save, E., Cressant, A., Thinus-Blanc, C., and Poucet, B. (1998). Spatial firing of hippocampal place cells in blind rats. J. Neurosci. *18*, 1818–1826.

Save, E., Nerad, L., and Poucet, B. (2000). Contribution of multiple sensory information to place field stability in hippocampal place cells. Hippocampus *10*, 64–76.

Scoville, W.B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20*, 11–21.

Shapiro, M.L., Tanila, H., and Eichenbaum, H. (1997). Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli. Hippocampus *7*, 624–642.

Sharp, P.E., and Koester, K. (2008). Lesions of the mammillary body region alter hippocampal movement signals and theta frequency: Implications for path integration models. Hippocampus *18*, 862–878.

Sharp, P.E., Kubie, J.L., and Muller, R.U. (1990). Firing properties of hippocampal neurons in a visually symmetrical environment: contributions of multiple sensory cues and mnemonic processes. J. Neurosci. *10*, 3093–3105.

Sharp, P.E., Tinkelman, A., and Cho, J. (2001). Angular velocity and head direction signals recorded from the dorsal tegmental nucleus of gudden in the rat: implications for path integration in the head direction cell circuit. Behav. Neurosci. *115*, 571–588.

Shinder, M.E., and Taube, J.S. (2011). Active and passive movement are encoded equally by head direction cells in the anterodorsal thalamus. J. Neurophysiol. *106*, 788–800.

Skaggs, W.E., and McNaughton, B.L. (1998). Spatial Firing Properties of Hippocampal CA1 Populations in an Environment Containing Two Visually Identical Regions. J. Neurosci. *18*, 8455–8466.

Skaggs, W.E., Knierim, J.J., Kudrimoti, H.S., and McNaughton, B.L. (1995). A model of the neural basis of the rat's sense of direction. Adv. Neural Inf. Process. Syst. *7*, 173–180.

Smith, D.M., Wakeman, D., Patel, J., and Gabriel, M. (2004). Fornix lesions impair

context-related cingulothalamic neuronal patterns and concurrent discrimination learning in rabbits (Oryctolagus cuniculus). Behav. Neurosci. *118*, 1225–1239.

Solstad, T., Boccara, C.N., Kropff, E., Moser, M.-B., and Moser, E.I. (2008). Representation of geometric borders in the entorhinal cortex. Science (80-.). *322*, 1865–1868.

Spiers, H.J., Hayman, R.M.A., Jovalekic, A., Marozzi, E., and Jeffery, K.J. (2015). Place field repetition and purely local remapping in a multicompartment environment. Cereb. Cortex *25*, 10–25.

Stackman, R.W., and Taube, J.S. (1997). Firing properties of head direction cells in the rat anterior thalamic nucleus: dependence on vestibular input. J. Neurosci. *17*, 4349–4358.

Stackman, R.W., and Taube, J.S. (1998). Firing properties of rat lateral mammillary single units: head direction, head pitch, and angular head velocity. J. Neurosci. *18*, 9020–9037.

Stackman, R.W., Clark, A.S., and Taube, J.S. (2002). Hippocampal spatial representations require vestibular input. Hippocampus *12*, 291–303.

Stackman, R.W., Golob, E.J., Bassett, J.P., and Taube, J.S. (2003). Passive transport disrupts directional path integration by rat head direction cells. J. Neurophysiol. 2862–2874.

Stackman, R.W., Lora, J.C., and Williams, S.B. (2012). Directional responding of C57BL/6J mice in the Morris water maze is influenced by visual and vestibular cues and is dependent on the anterior thalamic nuclei. J. Neurosci. *32*, 10211–10225.

Stensola, H., Stensola, T., Solstad, T., Frøland, K., Moser, M.-B., and Moser, E.I. (2012). The entorhinal grid map is discretized. Nature *492*, 72–78.

Stewart, S., Jeewajee, A., Wills, T.J., Burgess, N., and Lever, C. (2014). Boundary coding in the rat subiculum. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *369*, 20120514.

van Strien, N.M., Cappaert, N.L.M., and Witter, M.P. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nat. Rev. Neurosci. *10*, 272–282.

Tan, H.M., Bassett, J.P., O'Keefe, J., Cacucci, F., and Wills, T.J. (2015). The development of the head direction system before eye opening in the rat. Curr. Biol. *25*, 479–483.

Tanila, H. (1999). Hippocampal place cells can develop distinct representations of two visually identical environments. Hippocampus *9*, 235–246.

Taube, J.S. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. J. Neurosci. *15*, 70–86.

Taube, J.S. (2007). The head direction signal: origins and sensory-motor integration. Annu. Rev. Neurosci. *30*, 181–207.

Taube, J.S., Muller, R.U., and Ranck, J.B. (1990a). Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. J. Neurosci. *10*, 420–435.

Taube, J.S., Muller, R.U., and Ranck, J.B. (1990b). Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. J. Neurosci. *10*, 436–447.

Thompson, L.T., and Best, P.J. (1990). Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats. Brain Res. *509*, 299–308.

Thorndike, E.L. (1911). Animal intelligence (New York,: The Macmillan Company).

Tolman, E.C. (1948). Cognitive maps in rats and men. Psychol. Rev. *55*, 189–208.

Tolman, E., and Honzik, C. (1930). Introduction and removal of reward, and maze performance in rats. Univ. Calif. Publ. Psychol. *4*, 257–275.

Tolman, E.C., Ritchie, B.F., and Kalish, D. (1946). Studies in spatial learning 1: Orientation and the short-cut. J. Exp. Psychol. *36*, 13–24.

Tonegawa, S., Tsien, J.Z., McHugh, T.J., Huerta, P., Blum, K.I., and Wilson, M.A. (1996). Hippocampal CA1-region-restricted knockout of NMDAR1 gene disrupts synaptic plasticity, place fields, and spatial learning. Cold Spring Harb. Symp. Quant. Biol. *61*, 225–238.

Tsanov, M., Chah, E., Vann, S.D., Reilly, R.B., Erichsen, J.T., Aggleton, J.P., and O'Mara, S.M. (2011). Theta-modulated head direction cells in the rat anterior thalamus. J. Neurosci. *31*, 9489–9502.

Tulving, E. (1983). Elements of Episodic Memory (Clarendon Press).

Ulanovsky, N., and Moss, C.F. (2007). Hippocampal cellular and network activity in freely moving echolocating bats. Nat. Neurosci. *10*, 224–233.

Wallace, D.G., Gorny, B., and Whishaw, I.Q. (2002). Rats can track odors, other rats, and themselves: implications for the study of spatial behavior. Behav. Brain Res. *131*, 185–192.

Whishaw, I.Q., Hines, D.J., and Wallace, D.G. (2001). Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests. Behav. Brain Res. *127*, 49–69.

Wilson, M.A., and McNaughton, B.L. (1993). Dynamics of the hippocampal ensemble code for space. Science *261*, 1055–1058.

Wilton, L.A.K., Baird, A.L., Muir, J.L., Honey, R.C., and Aggleton, J.P. (2001). Loss of the Thalamic Nuclei for “Head Direction” Impairs Performance on Spatial Memory Tasks in Rats. Behav. Neurosci. *115*, 869–869.

Winter, S.S., Clark, B.J., and Taube, J.S. (2015). Disruption of the head direction cell network impairs the parahippocampal grid cell signal. Science (80-.). *347*, 870–874.

Witter, M.P. (1993). Organization of the entorhinal-hippocampal system: a review of current anatomical data. Hippocampus *3*, 33–44.

Witter, M.P., and Amaral, D.G. (1995). Hippocampal Formation. In The Rat Nervous System, G. Paxinos, ed. (Academic Press Inc), pp. 637–703.

Witter, M.P., Groenewegen, H.J., Lopes da Silva, F.H., and Lohman, A.H. (1989). Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Prog. Neurobiol. *33*, 161–253.

Wood, E.R., Dudchenko, P.A., Robitsek, R.J., and Eichenbaum, H. (2000). Hippocampal neurons encode information about different types of memory episodes occurring in the same location. Neuron *27*, 623–633.

Yartsev, M.M., Witter, M.P., and Ulanovsky, N. (2011). Grid cells without theta oscillations in the entorhinal cortex of bats. Nature *479*, 103–107.

Yoder, R.M., Clark, B.J., Brown, J.E., Lamia, M. V, Valerio, S., Shinder, M.E., and Taube, J.S. (2011). Both visual and idiothetic cues contribute to head direction cell stability during navigation along complex routes. J. Neurophysiol. *105*, 2989–3001.

Yoganarasimha, D., and Knierim, J.J. (2005). Coupling between place cells and head direction cells during relative translations and rotations of distal landmarks. Exp. Brain Res. *160*, 344–359.

Yoganarasimha, D., Yu, X., and Knierim, J.J. (2006). Head direction cell representations maintain internal coherence during conflicting proximal and distal cue rotations: comparison with hippocampal place cells. J. Neurosci. *26*, 622–631.

Yoganarasimha, D., Rao, G., and Knierim, J.J. (2011). Lateral entorhinal neurons are not spatially selective in cue-rich environments. Hippocampus *21*, 1363–1374.

Zhang, S., Schönfeld, F., Wiskott, L., and Manahan-Vaughan, D. (2014). Spatial representations of place cells in darkness are supported by path integration and border information. Front. Behav. Neurosci. *8*, 222.

Ziv, Y., Burns, L.D., Cocker, E.D., Hamel, E.O., Ghosh, K.K., Kitch, L.J., Gamal, A. El, and Schnitzer, M.J. (2013). Long-term dynamics of CA1 hippocampal place codes. Nat. Neurosci. *16*, 264–266.

Zugaro, M.B., Berthoz, A., and Wiener, S.I. (2001). Background, but not foreground, spatial cues are taken as references for head direction responses by rat anterodorsal thalamus neurons. J. Neurosci. *21*, RC154.